For Reference

NOT TO BE TAKEN FROM THIS ROOM

For Reference

NOT TO BE TAKEN FROM THIS ROOM

Ex libris
universitatis
albertensis

University of Alberta
Printing Department

Digitized by the Internet Archive
in 2019 with funding from
University of Alberta Libraries

https://archive.org/details/Wolff1966_0

THE UNIVERSITY OF ALBERTA

THE RELEASE OF NORADRENALINE FROM RAT HEARTS BY SYMPATHOMIMETIC AMINES

by

SHIRLEY A. WOLFF

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF PHARMACOLOGY

EDMONTON, ALBERTA

1966

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The Release of Noradrenaline from Rat Hearts by Sympathomimetic Amines," submitted by Shirley A. Wolff in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

To investigate whether the receptor pattern of the postulated amine carrier system in sympathetic nerve endings is similar to that of the α-adrenotropic receptor, the efficacy of a series of sympathomimetic amines as stimulators of α-receptors in β-blocked vascular smooth muscle (rabbit aorta loops) was compared with activity as releasers of ^{3}H-noradrenaline from isolated perfused rat hearts. An initial assumption in this work was that the uptake by the postulated amine carrier was the limiting factor for a sympathomimetic amine to release ^{3}H-noradrenaline stored in the nerve ending, i.e., the amine must first be taken up by the amine carrier system and then may exchange at the binding sites. Thus releasing activity was assumed to represent the affinity of the amine for the carrier system or uptake mechanism. In support of this assumption, it was found that cocaine and desmethylimipramine, which are known to block the uptake of amines into the nerve ending, blocked the releasing activity of ℓ-noradrenaline.

Several structure-activity relationships were observed within the series of sympathomimetic amines studied and a number of similarities between the effect of structural changes on α-receptor stimulation and releasing activity were noted. Thus there may be some similarity between the receptor pattern of these two active sites, but the α-receptor is much more selective.

In further support of the original assumption, correlation was noted between releasing activity and Iversen's data on inhibition of uptake, in which he expressed the % inhibition of uptake of ^{3}H-noradrenaline into isolated perfused rat hearts by various amines as the affinity of these amines for the carrier system. It is thus concluded that the limiting factor in release may be the uptake of the releasing agent into the nerve ending.

ACKNOWLEDGEMENTS

To Dr. C. W. Nash, my supervisor, I extend sincere appreciation for the invaluable assistance and helpful criticism which he has given me throughout the course of this work.

I wish to express my thanks to Mrs. Barbara Ferguson and Mr. Robert Freeman whose excellent technical assistance speeded the progress of the work.

I wish also to acknowledge with thanks the financial support of the National Research Council and the Medical Research Council of Canada.

The drugs used in these experiments were generously supplied by the following companies: Sterling-Winthrop (ℓ-noradrenaline bitartrate, d-noradrenaline bitartrate, ℓ-adrenaline bitartrate, d-adrenaline-ℓ-bitartrate, ℓ-isoprenaline-d-bitartrate dihydrate, d-isoprenaline-d-bitartrate), and Merck, Sharpe and Dohme (dℓ-metaraminol).

Table of Contents

Page

ABSTRACT i

ACKNOWLEDGEMENTS iii

LIST OF TABLES vi

LIST OF FIGURES vii

INTRODUCTION 1

LITERATURE REVIEW 3

A. Sympathomimetic Amines 3

B. Adrenotropic Receptors 6

C. Storage of Catecholamines 7

D. Uptake of Catecholamines 13

E. Mechanism of Drug-Induced Noradrenaline Release 18

METHODS 21

Section 1 - Experiments on Vascular Smooth Muscle 21

Section 2 - Release of ^{3}H-noradrenaline 22

RESULTS 27

A. Vascular Smooth Muscle 27

B. Release of Tritium from Rat Hearts 27

(i) Dose-Response Curve 27

(ii) Inhibitors of Drug-Induced Release 29

a. Cocaine 32

b. Desmethylimipramine 32

(iii) Controls 32

(iv) Drug-Induced Release 37

(v) The Amount of ^{3}H-Noradrenaline Released 42

DISCUSSION 46

Table of Contents (continued)

Page

SUMMARY AND CONCLUSIONS 57

BIBLIOGRAPHY 60

APPENDIX I 69

APPENDIX II 71

List of Tables

Page

I. Increase in the Rate of Release of Tritium Produced by Ten Sympathomimetic Amines 41

II. Amount of Tritium Released (Expressed as % of Amount in the Heart) 44

III. Summary: Ranking Order of Drug Action 45

Appendix II

1. Response of Vascular Smooth Muscle to ℓ-noradrenaline in the Presence of Propranolol (10 ng/mℓ of bath volume) ... 72

2. Responses of Vascular Smooth Muscle to Sympathomimetic Amines in the Presence of Propranolol (10 ng/mℓ of bath volume) 73

3. The Effect of Successively Increasing Doses of ℓ-Na, ℓ-A and dℓ-Met on the Release of Tritium from Isolated, Perfused Rat Hearts 74

4. Dose-Response Curves of ℓ-NA, dℓ-Met and ℓ-A on the Release of Tritium from Rat Hearts 76

5. The Effect of Cocaine HCl on the Release of Tritium Caused by ℓ-NA 77

6. The Effect of Desmethylimipramine HCl on the Release of Tritium Caused by ℓ-NA 78

7. Controls: Spontaneous Efflux of ^{3}H-noradrenaline 79

8. The Effect of ℓ-NA on the Release of Tritium from Isolated, Perfused Rat Hearts 80

9. The Increase in the Rate of Release of Tritium from Isolated, Perfused Rat Hearts by Ten Sympathomimetic Amines 82

10. The Increase in Release Rate of Tritium (% of the Amount in the Heart) Caused by Ten Sympathomimetic Amines 92

List of Figures

Page

1. Apparatus 23
2. Responses of Vascular Smooth Muscle to Sympathomimetic Amines in the Presence of Propranolol (10 ng/mℓ of bath) .. 28
3. The Effect of Successively Increasing Doses of ℓ-NA, ℓ-A and dℓ-Met on the Release of Tritium from Isolated, Perfused Rat Hearts 30
4. Dose-Response Curves of ℓ-NA, dℓ-Met and ℓ-A on Release of Tritium from Rat Hearts 31
5. The Effect of Cocaine HCl on the Release of Tritium Caused by ℓ-NA 33
6. The Effect of Desmethylimipramine HCl on the Release of Tritium Caused by ℓ-NA 34
7. Controls: Spontaneous Efflux of ^{3}H-noradrenaline 36
8. Effect of ℓ-NA on the Release of Tritium from Isolated, Perfused Rat Hearts 40
9. The Change in Rate of Tritium Efflux (as % of Amount in the Heart) Caused by 1 μM Doses of the Ten Sympathomimetic Amines 43
10. Influence of Structure on Releasing Activity 50

INTRODUCTION

Tracer studies with ^{3}H-noradrenaline have shown that sympathomimetic amines can cause a release of noradrenaline stored in the nerve endings (1, 2, 3), which may indicate some form of exchange with the naturally occurring mediator (4, 5). Preliminary studies (6) have shown that noradrenaline and adrenaline and their stereoisomers have different releasing activity and that there is probably a structure-activity relationship within a series of sympathomimetic amines.

It is well established that noradrenaline is rapidly taken up and stored in sympathetically innervated tissues (7, 8, 9, 10, 11, 12) and Dengler (8, 10) has given evidence that this uptake follows the kinetics of a saturable process. In addition, Iversen (13) has shown that the uptake of ^{3}H-noradrenaline into isolated perfused rat hearts can be inhibited by sympathomimetic amines and other agents and that this absorption reaction follows Michaelis-Menten enzyme kinetics. Thus these nerve endings may have in their membranes some form of carrier system for the transport of noradrenaline which can also act to absorb other sympathomimetic amines with varying degrees of efficiency (14). It is possible that the affinity for a membrane carrier and the subsequent uptake into the nerve endings is the controlling factor in the exchange and release of noradrenaline from nerve endings by sympathomimetic amines. If this is true the release of tritium from hearts labelled with ^{3}H-noradrenaline would be blocked by agents such as cocaine (15, 16) and desmethylimipramine (17, 18) which have been shown to block the uptake of ^{3}H-noradrenaline (13, 15, 16, 17).

The α-adrenotropic receptors of the various systems which respond to sympathetic stimulation are in close proximity to the nerve endings which release noradrenaline. It appears probable that the postulated carrier in the nerve endings competes with α-receptors for the available noradrenaline and may have a receptor pattern similar to that of the α-receptors. If these receptor patterns are similar, the ranking order of potency of a series of sympathomimetic amines as stimulators of α-receptors in vascular smooth muscle should bear some similarity to their potencies as releasers of tritium from isolated, perfused rat hearts in which the noradrenaline stores had previously been labelled with ^{3}H-noradrenaline. The object of this research is to determine whether the receptor pattern of the postulated noradrenaline carrier in sympathetic nerve endings is similar to that of the α-adrenotropic receptor.

LITERATURE REVIEW

A. Sympathomimetic Amines:

Over one hundred years ago, Vulpian demonstrated the presence in adrenal gland of a material which gave a green color with ferric chloride (19). He also showed that this material appeared in adrenal venous blood. Although he did not know it, he was probably demonstrating for the first time the storage and release of catecholamines. Subsequently, tissues which accumulate catecholamines have become known as chromaffin tissues because of their ability to show a characteristic histologic staining reaction due to the reduction of chromates (20).

The presence in adrenal medulla of pharmacologically active material and its release under conditions of stress (21) were well known before the end of the last century even though the chemical constitution of adrenaline was not established for another five years (22, 23). Only in the last 20 years have significant quantities of a second catecholamine, noradrenaline, been demonstrated in adrenal medulla (24, 25) and sympathetic nervous tissue (26). Although for a long time it had been argued that the material liberated from sympathetic nerve endings was not adrenaline but some chemically related compound (27) and although some shrewd speculation as to the nature of this transmitter material had been made, only relatively recently has it been established that the material liberated from sympathetic nerve endings is in fact noradrenaline (28).

Adrenaline and noradrenaline belong to a series of chemical compounds which evoke physiological responses similar to those exerted by sympathetic nerve stimulation. The term "sympathomimetic amines"

was introduced by Barger and Dale, 1910 (29), to characterize their action.

β-Phenylethylamine is the parent compound of the sympathomimetic amines, and consists of an aromatic nucleus, i.e., a benzene ring, and an aliphatic amine, ethylamine. The structure permits substitutions on the aromatic ring, the α- and β-carbon atoms, and the terminal amino group, to yield a great variety of compounds with sympathomimetic activity. Noradrenaline and adrenaline have OH groups substituted in the 3 and 4 positions of the benzene ring and an OH group substituted on the β-carbon atom; in addition, adrenaline has a CH_3 group substituted at the amino group, and is therefore a secondary amine.

Various workers have attempted to classify the sympathomimetic amines and have found that there exist qualitative as well as quantitative differences in the actions of the individual amines. Fleckenstein and Burn (30) studied the effect of denervation of the nictitating membrane of a large series of sympathomimetic amines. They divided these amines into three groups:

(a) amines which were less effective after denervation (substances with not more than one -OH group in the benzene ring and without an -OH group on the β-carbon atom).

(b) amines which were not much affected by denervation (substances with not more than one -OH group in the benzene ring, but having an -OH group on the β-carbon).

(c) amines which were potentiated by denervation (catecholamines).

Soon afterwards Fleckenstein and Bass (31) and Fleckenstein and Stöckle (32) reported that similar results could be obtained after the administration of cocaine and the same three groups could be distinguished. The amines of group (a) were suggested to act "indirectly" by releasing noradrenaline from its storage sites since "it was evident that their action on the normal membrane was likely to be connected with the presence of the transmitter at the nerve ending" (30). The catecholamines (group c), whose action was potentiated and not abolished after degeneration of the nerve fibres, were said to act directly at the receptor sites. Trendelenburg (33) introduced the term "amines with mixed action" to describe those of group (b). The sympathomimetic effects they produced appeared to be a combination of both direct and indirect actions.

After the discovery of the noradrenaline depleting action of reserpine by Carlsson (34) and the fact that this effect prevented the pressor response to tyramine (the prototype of the indirectly acting amines), the classification of the sympathomimetic amines was again studied. Trendelenburg and associates (35, 36) studied the influence of reserpine and cocaine on the action of sympathomimetic amines in an attempt to evaluate the relative degree of their direct, indirect and mixed actions. This group abolished reflex activity by using spinal cats and compared the modification of heart rate, blood pressure and nictitating membrane responses to sympathomimetic amines after short-term pretreatment with reserpine (35). They found that after short-term reserpine pretreatment (a large dose 24 hours before experimentation) noradrenaline stores are maximally depleted "as judged by the very small response of the nictitating membrane to nerve

stimulation" (33). However, supersensitivity to amines which act directly did not exist as after long-term pretreatment (small doses injected daily for 3, 7 or 14 days depending on the tissue to be sensitized) or after denervation and cocaine. Thus by use of animals rapidly depleted with reserpine, the possibility of underestimating an indirect response was reduced and these data were more reliable than tests for indirect action after denervation or long-term reserpine pretreatment where potentiation of a direct portion of the response could obscure part or all of the indirect effect. Although Trendelenburg's classification agreed quite well with that of Fleckenstein and Burn (31), this work showed that amines with mixed effects were not sharply separated from the other two groups but there was a graded transition from direct to indirect action. In addition it was concluded that hydroxylation of the benzene ring was a more important factor than hydroxylation of the β-carbon atom in increasing the direct acting properties and that the meta hydroxy compounds have more direct activity than the para compounds which, like tyramine, are more indirect acting. This had been noted by other authors as well (37).

B. Adrenotropic Receptors:

One of the more important factors in determining the effects of a sympathomimetic amine is that there are two types of receptor sites with which it can react to elicit a response in sympathetic effector cells. Dale (1906) was the first to differentiate two types of adrenergic receptors; one type on which adrenaline gave excitatory responses was "paralyzed" by ergot alkaloids, whereas the other on

which adrenaline elicited inhibitory responses was not (38). Ahlquist (1948) classified these receptors as α and β, on the basis of their responses to a variety of sympathomimetic amines and to adrenergic blocking agents (39). In general the effect on α-receptors is excitatory and that on β-receptors is inhibitory (exceptions: intestinal smooth muscle has α-receptors which are inhibitory and cardiac muscle has β-receptors which are excitatory).

Ariens (1960) investigated the effects of substituents on the phenyl radical or on the amino group of catecholamines and concluded that the intrinsic activity for action of catecholamines on α-receptors was especially related to the interaction of the amino group and α-receptor, while the action on β-receptors may be especially related to the interaction of the catechol group and β-receptor (40).

The adrenergic blocking action of many compounds, such as imidazolines and haloalkylamines, is selective for the α-receptors, whereas other blocking agents, for example, pronethalol and DCI act only on β-receptors. So selective are these blocking agents that it has become customary to identify the type of adrenotropic receptor in a tissue on the basis of the effect of the adrenergic blocking agents on the responses to sympathomimetic amines. It must be appreciated that most sympathomimetic amines influence both α- and β-receptors, but that the ratio of the α- and β-activity varies tremendously between drugs, in a continuous spectrum from an almost pure α-activity (phenylephrine) to an almost pure β-activity (isoprenaline).

C. Storage of Catecholamines:

Catecholamines had been found in both tissues and nerves by

various workers prior to and during the 1930's (27, 41, 42). Today it is well established that noradrenaline is contained in sympathetically innervated organs (e.g., heart, spleen and blood vessels) in relatively constant amounts. This may indicate that the storage capacity is limited. Goodall (43) has shown that the noradrenaline content of organs innervated by adrenergic nerves is unaffected when the preganglionic fibres are sectioned but disappears when the postganglionic fibres are sectioned and allowed to degenerate. These findings indicate that noradrenaline is stored chiefly in the adrenergic fibres in the organ. Also consistent with this is the fact that noradrenaline is present in various parts of the postganglionic fibre. More recently the histofluorescence methods of Falck (44) have strongly supported these earlier indications that the storage sites of peripheral noradrenaline are in the adrenergic nerves.

A considerable proportion of the catecholamines in the adrenal medulla were found by Blaschko and Welch (45) to be localized in subcellular particles, which can be isolated by differential centrifugation. Similar catecholamine containing particles were found in 1956 by von Euler and Hillarp (46) in noradrenergic axones (bovine splenic nerves) and in sympathetically innervated organs (rat spleen). These so-called "storage particles" also take part in the synthesis of noradrenaline. Storage granules isolated from adrenal medulla and from rat hearts have been shown to convert dopamine to noradrenaline (48) indicating the presence of the enzyme dopamine-β-hydroxylase.

Potter has recently (49) classified the vesicles from "tissues of neural origin" as revealed by electron microscopy into the following groups: synaptic vesicles, small granulated vesicles, large granulated

vesicles, neurosecretory granules and chromaffin granules. "Of these particles only small granulated vesicles have been shown to be associated with noradrenaline" (49).

Hillarp (50) has studied some of the biochemical properties of isolated catecholamine granules in the cow adrenal medulla and has measured their lipid, protein, adenine nucleotide and catecholamine content. The average amount of noradrenaline and other constituents in granulated vesicles is not known and a more accurate measurement awaits better purification of the vesicular fraction. However, the enzymic content of heart vesicles which have been partially purified has been determined and in both splenic nerve and heart vesicles, ATP has been demonstrated (51). "ATP has often been suggested as an ionic complexing agent responsible for concentrating amines within vesicles or as the final binding substance, or both" (49). Weiner and Jardetzky (52) have made a study of catecholamine nucleotide complexes and found that weak complexes exist in solution. The complex so formed may involve some third component, probably intragranular protein. According to Burack _et al._ (53) there is a certain deficit in total adenine nucleotides relative to the catecholamines in adrenal granules (0.3 to 1.1 M). When present in a complex their concentration may be isotonic with the tissue fluids but if these constituents were free their combined concentration would be hypertonic (53). ATPases which are activated by Mg^{++} or Ca^{++} have been demonstrated in noradrenaline-containing heart vesicles (54). Douglas (55) has shown that if the adrenal medulla is perfused with a Ca^{++} free Locke's solution there is little or no secretion of catecholamines. "It is tempting to speculate that stimulus-secretion coupling involves an ion-activated enzyme, like

ATPase, which either causes an abrupt disruption of a catecholamine binding complex or causes a sudden change in membrane permeability, or both" (49).

The concept that the noradrenaline store in sympathetically innervated tissues is non-homogeneous is supported by pharmacological evidence. "Compartments of noradrenaline have been suggested on the basis of differential drug responses (56, 57, 58), different uptake rates (11), different release rates and modes of metabolism (59, 60) and different specific activities of noradrenaline present in the heart at different times (61, 62)" (49).

The vasoconstrictor response to tyramine which is diminished or lost after reserpine treatment was only observed when the stores were largely depleted and could be temporarily restored by an intravenous infusion of noradrenaline, which caused no, or a barely, detectable increase in the tissue noradrenaline content (33). This implies that the tyramine sensitive pool may be quite small. Different pools of noradrenaline with different turnover rates have been indicated by studies done with radioactively labelled noradrenaline (63, 59, 60). Kopin _et al._ (59) found that in the isolated perfused heart ^{3}H-noradrenaline is rapidly taken up and when further perfused with ^{3}H-noradrenaline-free solution a "multiphasic decline" in noradrenaline content occurs, indicating release from more than one pool. This has also been demonstrated _in vivo_ by Costa _et al._ (60) in hearts of intact mice, rats and guinea pigs.

Stjarne (64) has shown that granules isolated from bovine splenic nerves rapidly take up ^{3}H-noradrenaline but the specific activity of the intragranular amines never reached that of the

extragranular amines. This would suggest that not all of the stored noradrenaline is readily exchanged. Potter *et al.* (58) found that if tyramine was administered 24 hours after ^{3}H-noradrenaline only unlabelled catecholamine is released (58, 65, 66), and the specific activity of ^{3}H-noradrenaline rises equally in the soluble and particulate fractions (67). These observations have encouraged general acceptance of the concept of non-homogeneity of the noradrenaline store in sympathetically innervated tissues.

Reserpine has proved to be a highly efficient pharmacological tool for analysis of the catecholamine stores. *In vivo*, reserpine administration causes a profound depletion of the noradrenaline stores of peripheral noradrenergic nerves leading to failure of the impulse transmission at the sympathetic neuro-effector junction (68). However, even the lowest *in vivo* dose of reserpine which has a full depleting action, has no direct amine releasing effects on the storage granules from bovine splenic nerves (69) or when in concentrations lower than 10^{-4} M, from rat heart (51). In fact, at very low concentrations reserpine strongly inhibits the spontaneously occurring outflow of amines from the storage granules into the external medium on incubation *in vitro*, both in nerve and adrenal medulla granules (69). If *in vivo*, reserpine has no releasing effect on the granules, then much of the noradrenaline must be stored elsewhere since reserpine can deplete the noradrenaline stores. "Therefore, there may be at least one extra-vesicular store of noradrenaline" (70). In some way it must be protected from the action of monoamine oxidase, thus it is not likely to free in solution (70).

Crout (71) has suggested that non-homogeneity of the noradrenaline

stores or compartmentalization need not be accounted for by the presence of different "bound forms" of noradrenaline or the existence of extra-axonal "non-specific" binding sites. He has postulated that "firmly-bound" ^{3}H-noradrenaline, which disappears slowly over a period of several days, is "derived largely from granules lying near the axonal membrane" and that ^{3}H-noradrenaline in deeper granules is lost mainly via intracellular metabolism by monoamine oxidase" (71). Pools may be the result of differences in the affinity of noradrenaline to several binding sites within a single vesicle or it may be that different binding sites are located in different types of vesicles (70).

Various derivatives of phenylethylamine can be taken up into noradrenaline storage vesicles as demonstrated by Mussacchio et al. (14) but "only β-hydroxylated or catecholamines are retained in these particles" (70). The importance of the β-hydroxyl group for retention by the noradrenaline storage granule is indicated by the differences in retention of tyramine and its β-hydroxylated derivative, octopamine. Mussacchio (14) found that when tyramine is injected in tracer doses, it rapidly disappears from the tissues so that none remains after one hour. Octopamine disappears much more slowly. The importance of the β-hyrdoxyl group in the binding mechanism is also suggested by the fact that binding of ℓ-noradrenaline considerably exceeds that of d-noradrenaline (11, 72, 73, 74) and when labelled dℓ-noradrenaline is injected, the ℓ-isomer is preferentially retained (75). Dopamine, which does not have a β-hydroxyl group, is retained in heart microsomes when disulfiram is used to inhibit β-hydroxylase (14). This phenomenon is apparently related to the catechol structure. The conversion of dopamine to noradrenaline by β-hydroxylase further

enhances the affinity for the binding sites. Sympathomimetic amines are retained less efficiently when they lack one of the three hydroxyl groups of noradrenaline (noradrenaline > octopamine > phenylethylamine). Dihydroxymandelic acid is not retained in storage vesicles, therefore the quaternary nitrogen group $(- \overset{|}{\underset{|}{N}} -)^{+}$ is essential for binding (70). However, the binding decreases with increasing N-substitution (noradrenaline > adrenaline >> N-dimethyl-noradrenaline; isoprenaline: nil).

Metaraminol and other α-methylated phenolethanolamine derivatives (α-methyl-adrenaline and octopamine) are well retained in storage vesicles, probably because of their resistance to monoamine oxidases rather than any affinity of the α-methyl group for the binding site. It appears that efficient binding may involve all of the chemical groups of noradrenaline; consistent with this is the fact that O-methylation abolishes binding in vesicles (76).

There appears to be an inverse relationship between the proportion of bound amine which is retained in the microsomal fraction in tissue homogenates and the ease of drug-induced release of these amines *in vivo* (14).

D. Uptake of Catecholamines:

The uptake of exogenous catecholamines into storage sites in certain peripheral tissues has been extensively studied in the last 30 years. Muscholl (77), Strömblad and Nickerson (78), and Campos and Shideman (79) demonstrated an increase in the noradrenaline content of rat and dog hearts after the administration of ℓ-noradrenaline *in vivo*. Hertting *et al.* (80), Kopin and Gordon (81), and Crout (71) performed similar studies of uptake using $d\ell$-^{3}H-noradrenaline. Noradrenaline

uptake has also been demonstrated in isolated perfused organs such as hearts or atria of the guinea pig, rabbit, dog and rat or cat spleen (12, 82, 83, 84, 85, 86).

Considerable evidence is now available to support the assumption that the uptake of catecholamines occurs into postganglionic sympathetic neurones. Tissues in which the postganglionic sympathetic nerve supply has been cut and allowed to degenerate lose the ability to accumulate exogenous noradrenaline (75, 87). Hertting and Axelrod (88) showed that ^{3}H-noradrenaline taken into the spleen could be released by stimulation of the splenic nerve, indicating that localization of the accumulated noradrenaline parallels that of the endogenous noradrenaline. Also consistent with this view is the fact that uptake does not occur in tissues of "immunosympathectomized" rats (89) and mice (90).

The study of subcellular localization of catecholamines has provided further evidence that ^{3}H-noradrenaline is accumulated in the sympathetic nerve endings. Potter and Axelrod (47) have shown that about 80% of the circulating ^{3}H-noradrenaline was taken up by a particulate fraction associated with the microsomes of the heart and salivary gland. Endogenous catecholamines were also found in this fraction of the cell. Autoradiographic and histochemical techniques have made it possible to visualize sympathetic nerves. Wolfe (91), who used a combination of autoradiography and electron microscopy, showed a striking localization of ^{3}H-noradrenaline in the preterminal regions of autonomic axons of the rat pineal gland. Marks *et al.* (92) showed the localization of ^{3}H-noradrenaline in the nerve fibres of mouse brain, spleen and heart using autoradiographic techniques.

Noradrenaline will fluoresce when treated with formaldehyde (it is converted to a 3,4-dihydroisoquinoline). This technique was used by Hamberger *et al.* (93) to visualize accumulation of exogenous noradrenaline in the sympathetic nerve plexus of the rat iris.

That uptake of exogenous noradrenaline occurs into the terminals of postganglionic sympathetic nerves is a generally accepted theory; however, Fischer and Kopin (94) have recently provided evidence in denervated rat salivary glands for an "extraneuronal uptake site."

The mechanism of the catecholamine uptake process remains obscure. Accumulation of labelled catecholamine in various tissues of the cat to a greater level than in the plasma was observed during the infusion experiments of Axelrod *et al.* (7) and Whitby *et al.* (9). Dengler *et al.* (8, 10) showed that incubated slices of cat heart or brain could accumulate ^{3}H-noradrenaline to "levels up to five times those in the incubating medium." Iversen (11) and Lindmar and Muscholl (12) found that in the isolated perfused rat heart noradrenaline accumulated to "levels 40 times those in the perfusing medium." Dengler *et al.* (8, 10) studied *in vitro* the uptake of $d\ell$-^{3}H-noradrenaline by slices of cerebral cortex, heart and spleen and by isolated pineal bodies of the cat and found that the uptake process was saturable. Iversen (11) found that when his uptake data were plotted in the form of the Michaelis-Menten equation as S/v against S they were found to agree well with classical enzyme kinetics for a saturable process. The K_m value for ℓ- and d-noradrenaline (2.66×10^{-7} and 13.9×10^{-7} respectively) showed that the physiologically occurring ℓ-noradrenaline had 3 - 4 times greater affinity for uptake. This had been observed earlier (95).

Iversen's work has indicated that there are two different catecholamine uptake processes depending on their concentration in the perfusion medium. When catecholamine levels higher than those required to saturate the uptake process (about 1 μg noradrenaline/ml) are used a striking increase in catecholamine uptake occurred. This process was designated by Iversen (96) as Uptake$_2$. The two uptake processes differ markedly. For example, Uptake$_2$ shows no preference for the levo isomer of noradrenaline but does prefer adrenaline to noradrenaline and catecholamines taken up by this process are rapidly washed out with catecholamine-free medium (half-time 2 - 3 min), whereas with the first uptake process negligible wash-out occurs. Uptake$_2$, however, is also saturable, "but only at noradrenaline and adrenaline concentrations of 85 μg per mℓ and 20 μg per mℓ respectively" (96).

Iversen (97, 98, 99) has tested a large number of amines as inhibitors of Uptake$_1$ by measuring the rate of ^{3}H-noradrenaline uptake in the rat heart from a perfusion medium containing noradrenaline and the amine to be tested. The affinity of the drug for the uptake site was determined by measuring the concentration of the drug which would produce a 50% inhibition (ID_{50}) of noradrenaline uptake. Structurally similar amines probably inhibit uptake by competing with noradrenaline for the uptake site. "This method does not discriminate between drugs which inhibit noradrenaline uptake and are themselves transported into the tissue, and drugs which inhibit the uptake mechanism without themselves being transported" (99).

Burgen and Iversen from these data were able to describe to some extent the structural specificity of the catecholamine uptake

site and found the following structure-activity relationships (98):

(a) β-hydroxylation produced a decreased affinity for the uptake site. In such compounds the L-enantiomer had a higher affinity than the D-enantiomer.
(b) α-methylation resulted in a considerable increase in affinity for the uptake site; again this effect was stereochemically specific--in this case the D-enantiomer having considerably more activity than the L-enantiomer.
(c) Phenolic hydroxyl groups enhanced the affinity for the uptake site, *para*- and *meta*-substitutions having approximately equal effects. The optimal structure was the 3,4-dihydroxyphenyl group.
(d) N-substitution decreased the affinity of the drug for the uptake site. This effect was dependent on the size of the N-substituent, bulky substituents having correspondingly greater effects in depressing affinity.
(e) O-methylation of phenolic hydroxyl groups produced a striking decrease in affinity for the uptake site. *Meta*-methoxy compounds had considerably lower affinities than the corresponding *para*-methoxy amines.
(f) The phenethylamine structure could be replaced by saturated five and six membered ring structures as in propylhexedrine and cyclopentamine without producing a marked decrease in affinity for uptake.

These rules were well obeyed by the phenylethylamine series of compounds.

Other drugs, bearing no structural resemblance to each other, were also tested as inhibitors of Uptake_1. Cocaine and desmethylimipramine were found to be potent inhibitors. Cocaine produced a simple inhibition but that produced by desmethylimipramine appeared more complex as it progressed with time.

Shore *et al.* (100) have shown that ℓ-metaraminol is readily accumulated in the rat heart. Iversen found that ℓ-metaraminol was the most potent of the amines tested as inhibitors of noradrenaline uptake. It may be that other amines with a high affinity for the uptake site are also taken up into the tissue. Burn and Rand (116) were among the first to suggest that indirectly acting amines like

tyramine act by releasing noradrenaline from its storage sites and "an uptake into adrenergic terminals may be a necessary prelude to the subsequent displacement" (99). Drugs which inhibit catecholamine uptake, e.g., cocaine, may be acting in this way to abolish the pharmacological effects of indirectly acting amines. The uptake of direct acting amines into the tissue may serve as a mechanism for inactivation. It is generally assumed that binding is quantitatively a more important mechanism for the inactivation of noradrenaline than is enzymatic O-methylation.

The uptake of noradrenaline has also been extensively studied in slices (6, 10) and in subcellular particles (64). These studies also indicate the existence of a certain degree of structural specificity both in uptake and storage mechanisms. Several substances have been found to inhibit uptake of noradrenaline into slices and particles; these include reserpine, structural analogues of noradrenaline and substances like imipramine (10, 64).

E. Mechanism of Drug-Induced Noradrenaline Release:

Much of the present knowledge of noradrenaline-releasing sympathomimetic amines depends on experimental work with tyramine. Intravenous injections of tyramine increase the amount of noradrenaline in the venous outflow of the heart *in vivo* (1) and in the effluent of isolated perfused hearts (77). Tyramine increases the spontaneous release of noradrenaline from isolated bovine splenic nerve granules (101) and releases catecholamines from isolated bovine adrenal medullary granules by replacing the endogenous amines stoichiometrically (102). The mechanism involved in the release of noradrenaline is not yet known, but the fact that

storage granules from the adrenal medulla take up tyramine in vitro while noradrenaline is being released (102), suggests the possibility of a competitive displacement.

The depletion of noradrenaline from isolated granules by tyramine was absent at 0°C (102) and was completely inhibited by reserpine at low concentration (103). von Euler and Lishajko (69) also observed that low concentrations of reserpine strongly counteract the spontaneous release of amines from the isolated nerve granule. Spontaneous release is also absent at 0°C (102). Thus an alternative hypothesis may be that tyramine is competing with noradrenaline spontaneously released for uptake into the granules. "Many other noradrenaline releasers may similarly act by competitively inhibiting re-uptake into the granules of noradrenaline spontaneously released from them" (104).

Metaraminol and other hydroxylated phenylethylamines like α-methylnoradrenaline and octopamine displace noradrenaline and are themselves taken up and stored as false neurotransmitters (106). These drugs can be released by reserpine (105, 106) and can be released from heart by sympathetic nerve stimulation (105). The mechanism of release is not known; these drugs may in some way affect the permeability of the presynaptic terminal (106, 107, 108).

Udenfriend (109) found that when metaraminol was used to release noradrenaline the catecholamine level in the tissue remained about the same. The lost noradrenaline was compensated for by the metaraminol taken up. Thus stoichiometric displacement

has been the general assumption.

METHODS

Section 1: Experiments on Vascular Smooth Muscle.

New Zealand white rabbits weighing between 1.5 and 2.0 kg. were killed by a blow on the head. The thoracic aorta was rapidly removed, care being taken to prevent stretching. Fat and connective tissue were trimmed off and the aorta was cut into loops 3 mm wide. Four loops were suspended in 50 mℓ of Krebs-bicarbonate solution (Appendix I) in the muscle chamber and aerated with 95% O_2 and 5% CO_2. Two grams tension was applied to each tissue. The muscle loops were allowed to relax for 2 or 3 hours after suspension in the organ bath with the tension maintained at 2 grams. After the responses of the tissue to a test dose of ℓ-noradrenaline had become reproducible, experimental observations were begun.

The contractile response was measured by use of Grass strain gauge transducers and a Grass polygraph.

All drugs were allowed to remain in contact with the tissues for 3 minutes. To block β-receptor activity, propranolol was used in a concentration of 10 ng/mℓ of bath medium and was allowed to remain in contact with the muscle for 3 minutes prior to addition of drug. The bath was drained and propranolol was readministered along with the drug and a fresh medium.

Dose-response curves were determined for the drugs studied. All comparisons were made on the basis of the molar concentration of test drugs and plotted as percentages of the estimated maximum response to ℓ-noradrenaline. The maximum response to ℓ-noradrenaline was estimated by plotting the double reciprocal dose-response curve (110) and

extrapolating to the origin. These estimates were checked by plotting the response vs. the response/the dose, i.e., the Eadie method (111, 112, 113), which gave very good agreement. (Appendix II, Table 2.)

Section 2: Release of ^{3}H-noradrenaline.

A. Preparation and Perfusion of the Heart:

Male Sprague-Dawley rats weighing between 150 and 200 grams were killed by a blow on the head. The heart was removed, cannulated via the aorta with a blunted 18-gauge needle and perfused by a modified Langendorff method at a flow rate of 4 mℓ/min. The flow was maintained constant by use of a Harvard peristaltic pump. Krebs-bicarbonate solution, pH 7.4, equilibrated with 95% O_2, 5% CO_2 was used in all experiments and had attained a temperature of 37° C in a heating coil immediately before entering the heart (Figure 1). Four hearts were perfused simultaneously. The effluent was collected in 50 mℓ centrifuge tubes which were changed at 3 or 6 minute intervals according to the program for the experiments.

The labelled noradrenaline and test drugs were added to the perfusion fluid just before entry to the heart by infusion at a constant rate from an infusion pump, consisting of a motor driven syringe. The dose administered could be varied by changing the speed of the infusion pump.

dℓ-noradrenaline-7-^{3}H (S.A. = 4.27 c/mMole) obtained in chromatographically pure form from New England Nuclear Corporation, was diluted first with 0.01 N HCl (see Appendix I) and then with acid Krebs solution (prepared without glucose or bicarbonate) such that when infused the final concentration entering the heart was 4.2 ng ^{3}H-noradrenaline

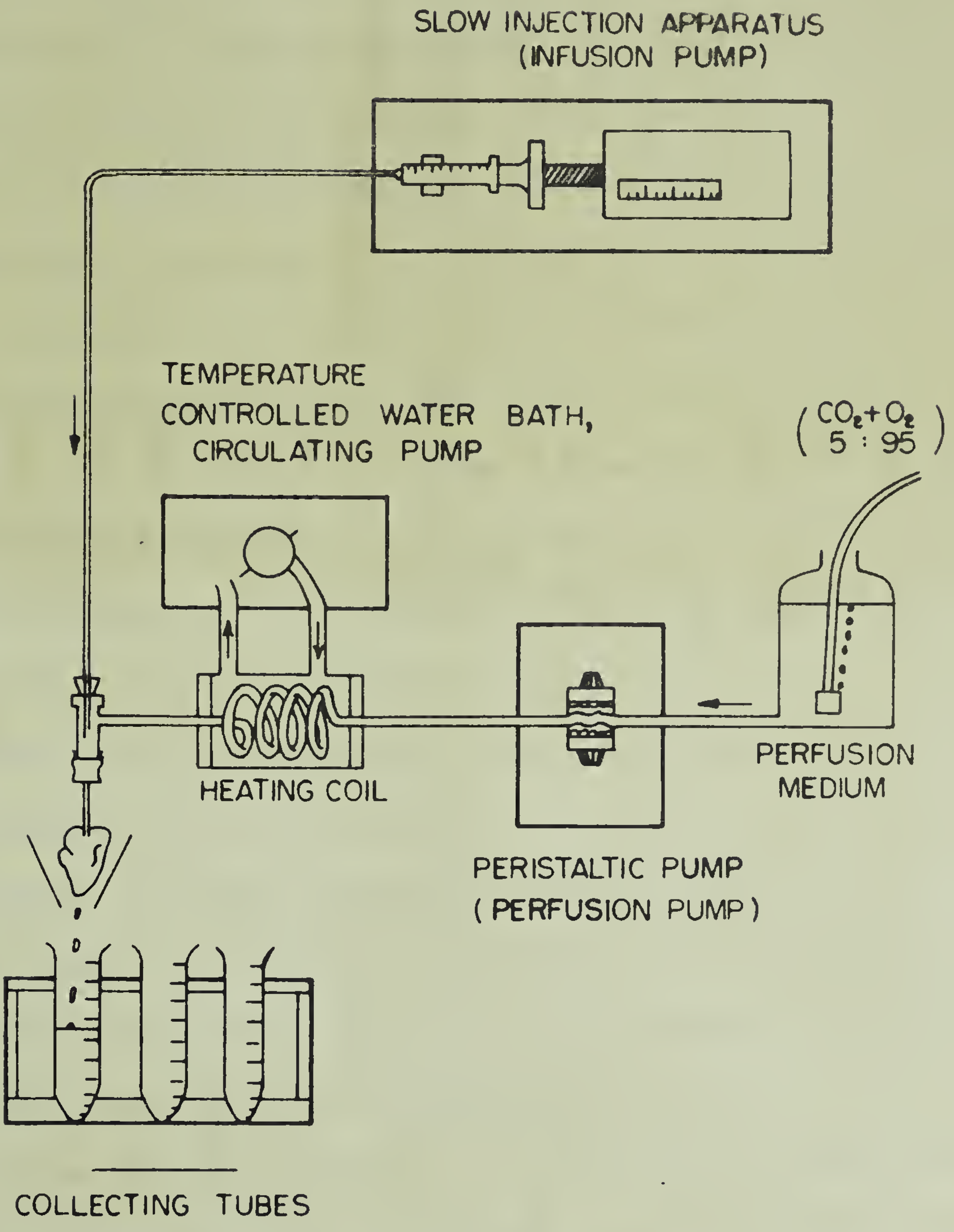
FIGURE 1

Apparatus

Isolated rat hearts are perfused by the Langendorff method at a constant rate, with Krebs-bicarbonate solution equilibrated with CO_2 and O_2 (5:95) and heated to 37° C. Radioactive materials and drugs were infused into the perfusion fluid at a controlled rate, by use of a motor driven syringe with a variable speed control, and mixed in a low volume T tube immediately above the heart. The slow injection apparatus operates 4 individual syringes and the perfusion pump acts on 4 separate plastic tubes so that 4 hearts are perfused simultaneously by this apparatus.

FIGURE 1

Apparatus



per mℓ per min.

To label the noradrenaline content of the hearts, ^{3}H-noradrenaline (4.2 ng/mℓ/min) was perfused through the hearts at a constant rate for 9 min (three 3-minute collection periods). The infusion pump was then disconnected and perfusion continued with Krebs-bicarbonate solution for 39 minutes, when the rate of spontaneous release of radioactive substances was relatively low and the rate of drug induced release could rise well above the variation in the rate of spontaneous release. When testing the releasing action of drugs, three 9-minute test periods were performed starting at the end of the 39-minute efflux period. In control experiments the hearts were perfused with Krebs-bicarbonate solution for 129 minutes after the cessation of ^{3}H-noradrenaline infusion.

Stock solutions of the drugs in 0.01 N HCl (Appendix I) were diluted with acid Krebs such that when infused and diluted with perfusion medium, the solution entering the heart was 1 μM. Tests indicated that the buffering capacity of the perfusion medium was great enough that the acid entering it did not appreciably alter the pH.

When blocking agents were used, the drug was dissolved directly in the perfusion medium and perfused for 18 minutes prior to administering test drugs. In these experiments, the test drugs were added over a 27-minute interval (9 minutes at each of 0.5, 1.0, and 2.0 μM concentrations) to obtain a dose-response curve, with the drug being tested as a blocking agent being present throughout this period.

To estimate radioactivity, a 1 mℓ aliquot of each sample collected was dissolved in 10 mℓ of phosphor solution containing 0.4% of 2,5-diphenyloxazole (PPO), 0.01% of p-bis-2'-(5'-phenyloxazolyl)benzene (POPOP), and 10% naphthalene in p-dioxane and assayed by scintillation

counting in a Nuclear Chicago Liquid Scintillation Counter. The background, which was determined by counting over 100 samples containing only scintillation fluid, was automatically subtracted during counting operations. Under the conditions of these experiments counting efficiency was calculated to be approximately 12% of the theoretical disintegration per minute (DPM). Although some quenching undoubtedly occurred, the conditions within and between the experiments were considered to have been sufficiently constant that the comparisons made were valid.

At the end of the experiment, the hearts were quickly removed from the perfusion apparatus, sliced longitudinally, blotted dry and frozen in liquid nitrogen. The tissue was kept in the frozen state until extraction procedures were carried out.

B. Estimation of Catecholamines in the Heart:

The catecholamines were extracted from the frozen tissue into 0.01 N HCl by the butanol-extraction method of Cass and Callingham (114).

Procedure:

The frozen tissue was weighed, disrupted in a stainless steel chamber designed by Cass (115) and homogenized in a glass homogenizer with 10 mℓ of chilled, acidified and salt-saturated n-butanol (see Appendix I). The homogenate was transferred to a stoppered centrifuge tube, shaken for 5 minutes and centrifuged at 2000 rpm for 10 minutes. (0.25 mℓ of the butanol layer and 0.75 mℓ of Krebs solution were added to 10 mℓ of phosphor solution for counting. The extraction of the ^{3}H-noradrenaline from the tissue into the butanol layer was also checked

by heating the sediment consisting of heart tissue with 2 mℓ 5 N NaOH on a water bath for 30 minutes and 0.2 mℓ of this digest in 0.8 mℓ of Krebs solution was counted.)

The supernatant butanol layer was transferred to a stoppered centrifuge tube containing 15 mℓ of n-heptane and 4.9 mℓ 0.01 N HCl. This mixture was shaken for 5 minutes and centrifuged at 2000 rpm for 5 minutes. (0.5 mℓ of the top organic layer in 0.5 mℓ of Krebs solution was counted.) The top organic layer was removed by aspiration and a 0.5 mℓ aliquot of the acid extract in 0.5 mℓ of Krebs solution was counted for its radioactive content. The radioactivity was assumed to be present in the form of ^{3}H-noradrenaline.

The Standard Errors of the means of the release at 1 μM concentration of the drugs were calculated using the following formula:

$$SE = \sqrt{\frac{\Sigma x^2 - (\Sigma x/n)^2}{n(n-1)}}$$

The Students Paired t-test was used to calculate the significance of the difference between the two drugs which were being compared.

$$t = \frac{\Sigma(x_1 - x_2)}{n} \Bigg/ \sqrt{\frac{\Sigma(x_1 - x_2)^2 - \left(\frac{\Sigma x_1 - \Sigma x_2}{n}\right)^2}{n(n-1)}}$$

P values were obtained from Fisher's distribution tables.

RESULTS

A. Vascular Smooth Muscle:

The contractile responses of rabbit aorta tissue to increasing doses of sympathomimetic amines were plotted in the log dose-response manner and gave the typical sigmoid curves. The tissues were treated with 10 ng/mℓ of propranolol, a β-receptor blocking agent, before and during each test in order to obtain a better measure of the effect of the test drug on the α-receptor alone. To facilitate comparisons of the relative potencies of the drugs, dose-response tests were made with ℓ-noradrenaline on the tissues used for the test drugs, and the responses to the test drugs were calculated as percentages of the maximum response to ℓ-noradrenaline. The maximal response to noradrenaline was estimated by preparing double-reciprocal dose-response plots (Appendix II, Table 2) and extrapolating to the origin.

Figure 2 shows the dose-response curves plotted from the data in Appendix II, Table 1. The resulting order of potency of the sympathomimetic amines studied as α-receptor stimulators is: ℓ-A > ℓ-NA > d-A = dℓ-Met > d-NA > ℓ-INA > Dopamine > Tyramine. Phenylethylamine and d-INA gave no response in the dose range used.

B. Release of Tritium from Rat Hearts:

(i) Dose-Response Curves.

The noradrenaline in the isolated perfused rat hearts was labelled by infusing ^{3}H-noradrenaline and the hearts were further perfused with Krebs-bicarbonate solution. When the efflux of tritium was counted and plotted, the multiphasic efflux curve showed a rapid outflow

FIGURE 2

Responses of Vascular Smooth Muscle to Sympathomimetic Amines in the Presence of Propranolol (10 ng/mℓ of bath).

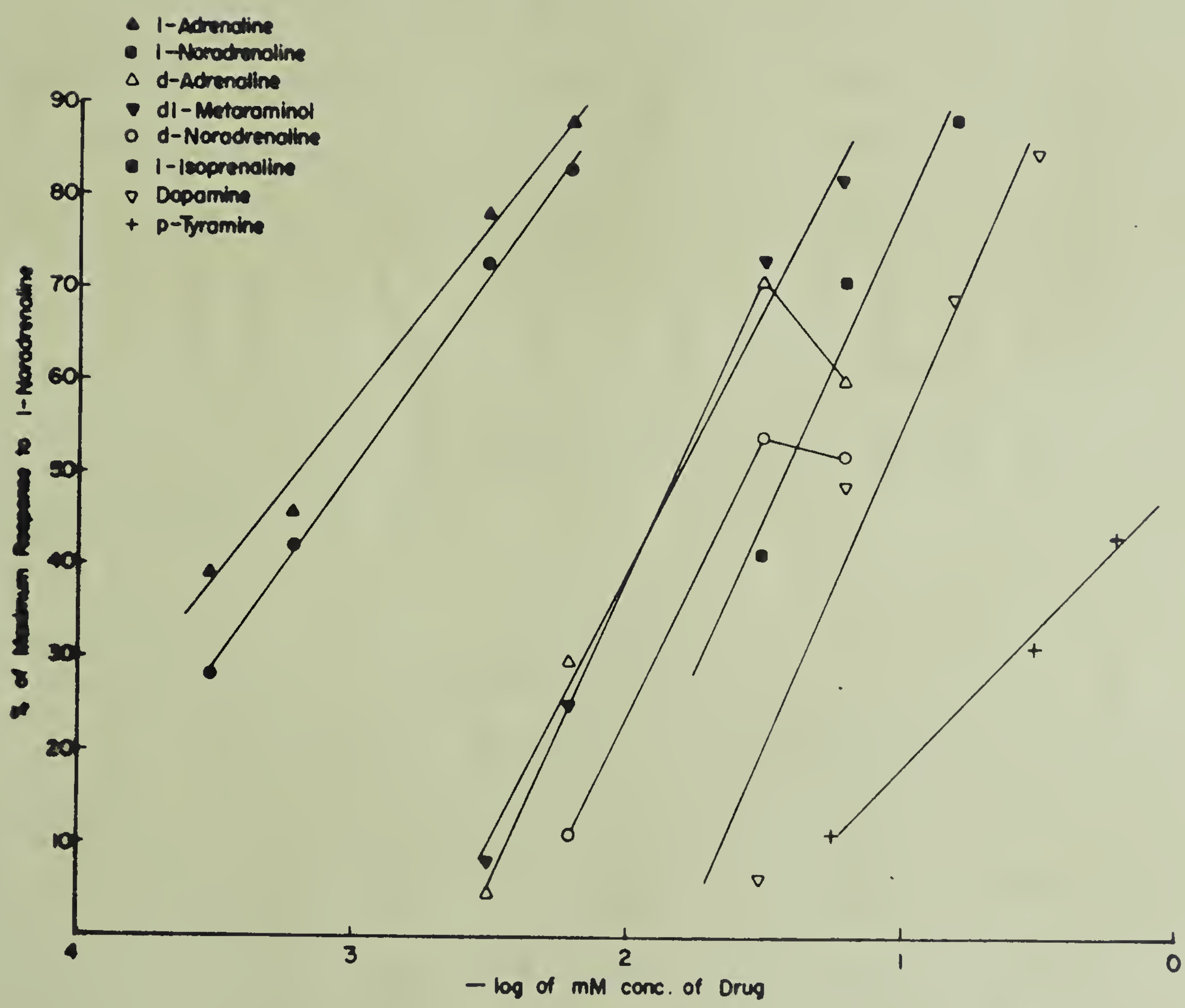
The contractile responses of rabbit aorta tissues to increasing doses of sympathomimetic amines are shown. The responses were expressed as a percent of the calculated maximum response to ℓ-noradrenaline.

The corresponding data are found in Appendix II, Tables 1 and 2.

Note: The responses to ℓ-isoprenaline were in the presence of 1 μg/mℓ of propranolol.

FIGURE 2

Responses of Vascular Smooth Muscle to Sympathomimetic Amines in the Presence of Propranolol (10 ng/ml of bath)



of tritium followed by a much slower outflow of the isotope, as indicated in the control curve of Figure 3. In order to determine drug effects on the release of tritium, the test drugs were infused after the fast phase of tritium efflux had passed, i.e., after 45 minutes of perfusion. The drugs tested, ℓ-NA, ℓ-A and dℓ-Met, were administered over a 27-minute interval in which the dose was increased stepwise; e.g., 9 minutes of perfusion with 0.5 μM, 9 minutes with 1.0 μM, and 9 minutes with 2.0 μM concentrations of the test drug. The increase in the rate of efflux of tritium caused by these drugs is also shown in Figure 3.

When the increase in the rate of efflux was calculated as a percentage of the amount in the heart at that time, a dose-response curve as shown in Figure 4 was obtained. These data show that for each of these drugs, the 1 μM dose induced approximately the maximum response but was not supermaximal, as increasing the dose to 2 μM produced a small additional increase in release rate. Thus, in further experiments, the 1 μM dose was chosen when comparing the relative releasing activity of the sympathomimetic drugs.

(ii) Inhibitors of Drug-Induced Release.

If the increase in release of ^{3}H-noradrenaline caused by sympathomimetic amines requires first an uptake into the nerve endings and then an exchange process at storage sites in the nerve ending, drugs such as cocaine and desmethylimipramine which block uptake into the nerve endings (16, 17) should cause an inhibition of release. To test this assumption cocaine and desmethylimipramine were tested for their ability to block the release of ^{3}H-noradrenaline caused by a noradrenaline infusion.

FIGURE 3

The Effect of Successively Increasing Doses of ℓ-NA, ℓ-A and dℓ-Met on the Release of Tritium from Isolated, Perfused Rat Hearts.

The control curve shows the rate of loss of radioactivity from 4 isolated perfused rat hearts during the 99 minutes following cessation of ^{3}H-noradrenaline infusion.

In all experiments the perfusates were collected for 3-minute intervals and each point represents the mean rate of release during that collection period.

The ordinate shows the mean rate of tritium release from the heart, at the midpoint of the interval, expressed as counts/min tritium leaving the heart per minute.

The releasing effects of 0.5, 1.0 and 2.0 μM concentrations of ℓ-NA, ℓ-A, and dℓ-Met are shown in this figure. Each value represents the mean rate of release from 4 hearts. The corresponding data are found in Appendix II, Table 3.

FIGURE 3

The Effect of Successively Increasing Doses of ℓ-NA, ℓ-A and dℓ-Met on the Release of Tritium from Isolated, Perfused Rat Hearts.

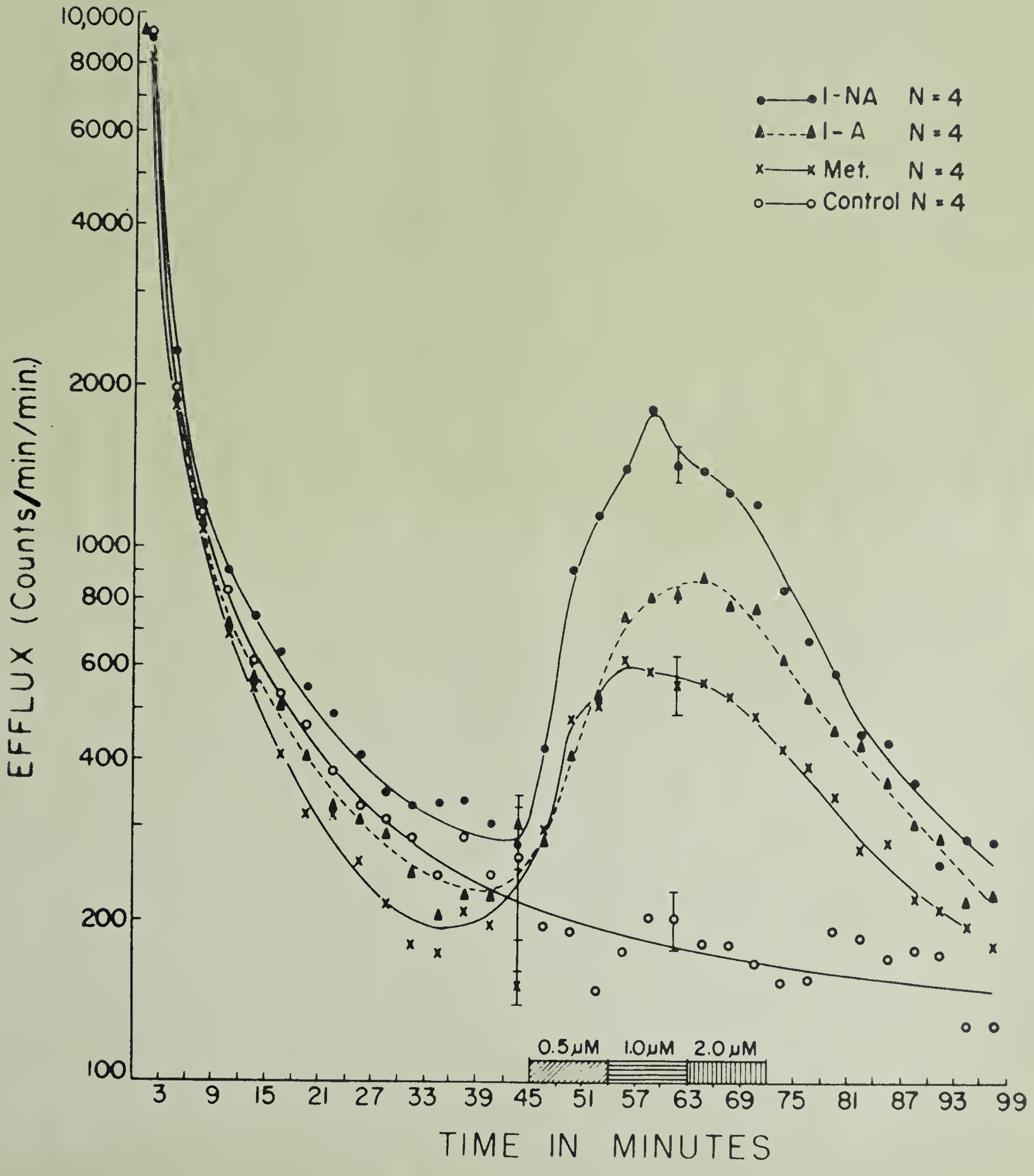


FIGURE 4

Dose-Response Curves of ℓ-NA, dℓ-Met, and ℓ-A on Release of Tritium from Rat Hearts.

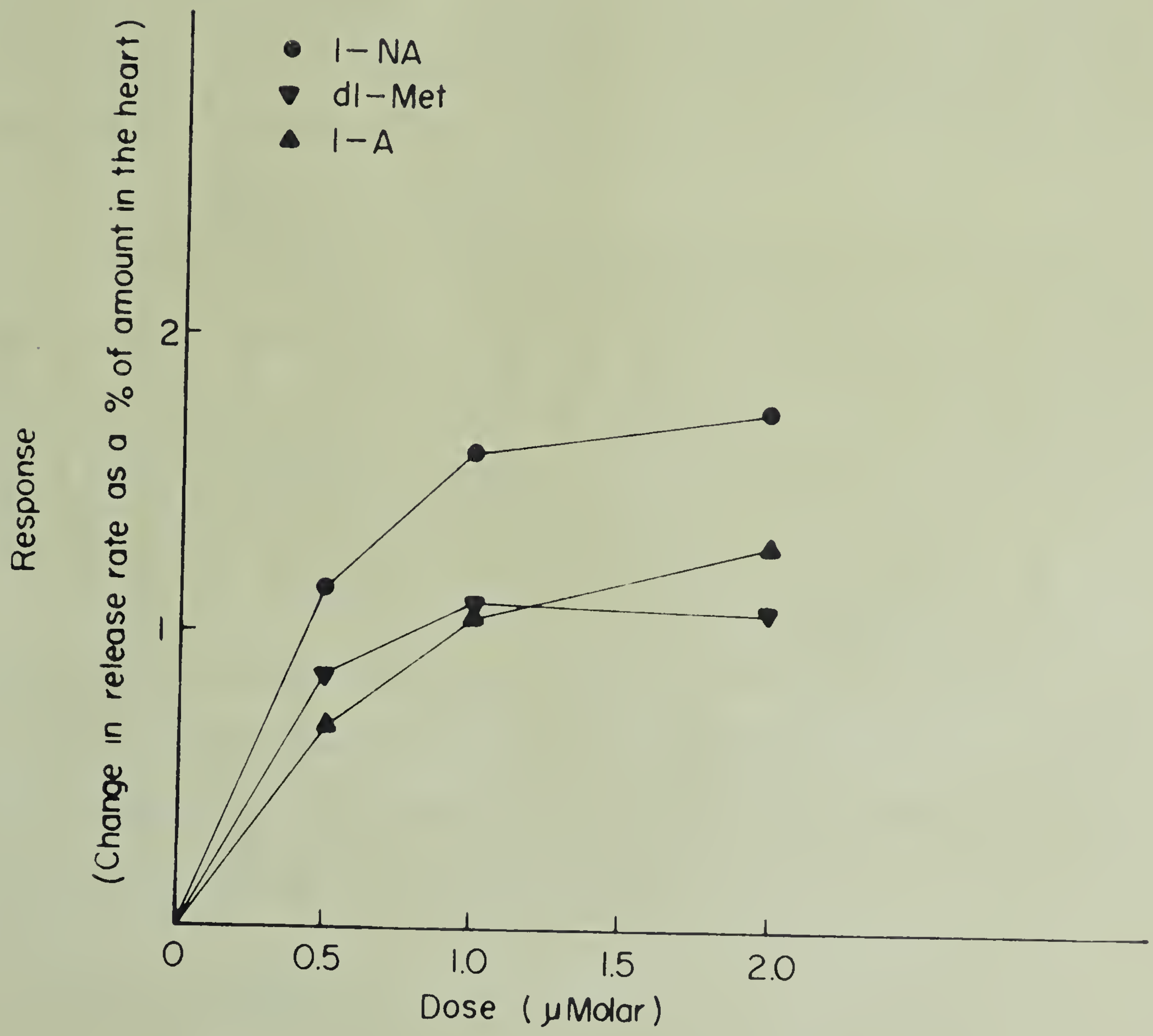
The rate of tritium efflux was calculated as a percentage of the amount in the heart at the time ($\frac{\text{efflux (c/m/m)}}{\text{Cum. c/m}}$ x 100) starting from the period before drug administration and continuing until after the highest dose (2.0 μM) had been given.

To calculate the change in the rate of efflux due to the infusion of a drug, the rate of efflux during the collection period just prior to starting the drug infusion was subtracted from each subsequent efflux rate. The average of the last two collection periods for each dose was plotted to give the dose-response curves shown. The 1 μM dose is shown to cause approximately the maximum response.

The corresponding data are in Appendix II, Table 4.

FIGURE 4

Dose-Response Curves of ℓ-NA, dℓ-Met and ℓ-A on Release of Tritium from Rat Hearts.



a. Cocaine.

Cocaine HCl was administered along with the Krebs-bicarbonate perfusion medium for a period of 18 minutes prior to, and throughout the infusion of ℓ-noradrenaline. At the concentrations used cocaine had no effect on the spontaneous release rate and, as Figure 5 illustrates, during the 18-minute interval prior to ℓ-noradrenaline infusion, the efflux curve followed its normal course. Increasing the dose of cocaine from 10^{-6} M to 10^{-5} M caused a marked reduction in the release of tritium caused by ℓ-noradrenaline. In the presence of cocaine, only the 2 μM concentration of ℓ-noradrenaline in the perfusion fluid produced a release and that release was very small.

b. Desmethylimipramine.

Figure 6 shows that when desmethylimipramine (10^{-6} M) was added to the perfusion medium beginning 18 minutes prior to drug infusion, it caused a small transient increase in the rate of tritium efflux but it blocked the release caused by ℓ-noradrenaline at all but the high concentration (2 μM), which was much reduced.

The fact that both cocaine and desmethylimipramine blocked release at low concentrations but that this block was partially overcome at higher concentrations of ℓ-noradrenaline suggests that the blockers and the ℓ-noradrenaline compete for a common site of action, perhaps a noradrenaline carrier in the nerve ending.

(iii) Controls.

Control efflux curves were obtained by first infusing 8 hearts with ^{3}H-noradrenaline to label their noradrenaline content in the manner described in the Methods section, and then continuing the perfusion with

FIGURE 5

The Effect of Cocaine HCl on the Release of Tritium Caused by ℓ-NA.

ℓ-noradrenaline (•—• ℓ-NA Control) caused an immediate increase in the rate of release of tritium which soon reached and maintained a maximum until the end of the infusion period. When 10^{-6} M Cocaine HCl was administered along with the perfusion medium, the effect of ℓ-NA was somewhat reduced, but when the Cocaine concentration was increased to 10^{-5} M it almost abolished the effect of ℓ-NA. At that concentration of Cocaine only the 2 μM dose of ℓ-NA produced an effect, and that effect was extremely small.

The corresponding data are in Appendix II, Table 5.

FIGURE 5

The Effect of Cocaine HCl on the Release of Tritium Caused by ℓ-NA.

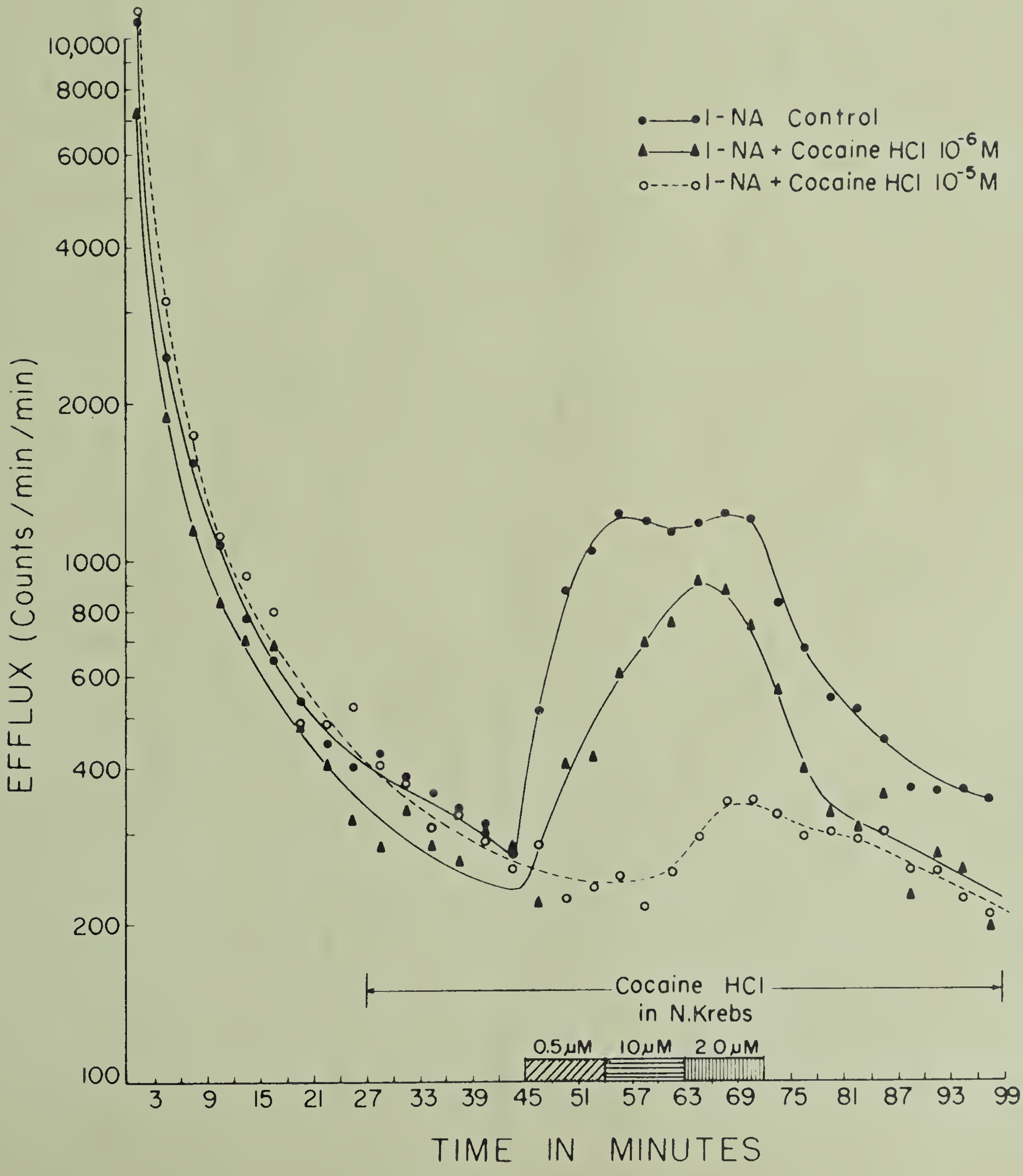


FIGURE 6

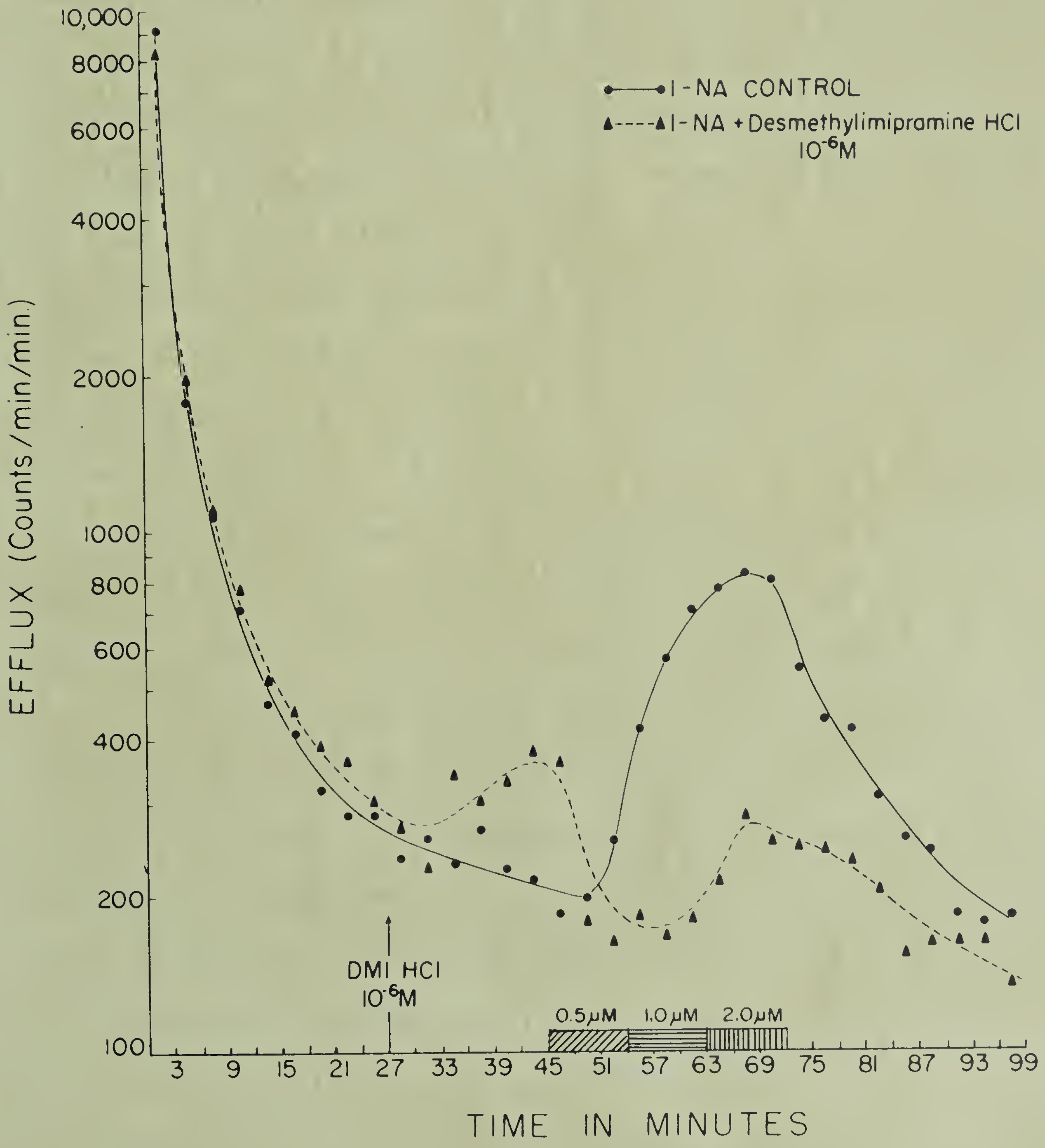
The Effect of Desmethylimipramine HCl on the Release of Tritium Caused by ℓ-NA.

When 10^{-6} M desmethylimipramine HCl was administered along with the perfusion medium, it caused a small transient increase in the release of tritium but blocked the releasing effect of 0.5 μM and 1.0 μM concentration of ℓ-NA and greatly reduced the effect of the 2.0 μM concentration of ℓ-NA.

The corresponding data are in Appendix II, Table 6.

FIGURE 6

The Effect of Desmethylimipramine HCl on the Release of Tritium Caused by ℓ-NA.



Krebs-bicarbonate solution at a rate of 4 mℓ/min for a further 129 minutes. To plot the curves, the means of all points at corresponding times were calculated.

The lower curve in Figure 7 shows the rate of loss of radioactivity from these 8 hearts during the 129 minutes following cessation of ^{3}H-noradrenaline infusion. The perfusates were collected for varying intervals (3 or 6 minutes). The total radioactivity leaving the heart during an interval was determined and the mean amount of tritium released per minute was calculated and expressed as the rate of loss of tritium (counts/min/min) during that interval. This was plotted at the midpoint of the interval. By adding the amount of tritium (counts/min) released from the hearts during each sample period to the amount present in the heart at the end of the experiment, the total amount of tritium in the heart at any time can be determined and is shown in the cumulative counts curve (upper curve) in Figure 7. This curve shows the decline of total radioactivity in the heart plotted as counts/min against time, and these experiments reproducibly showed an initial rapid exponential decline followed by a slower multiphasic decline.

If the change in the rate of efflux is due to outflow from different compartments in the tissue and the initial rapid decline represents the efflux of ^{3}H-noradrenaline from the intravascular and interstitial spaces, then the slower multiphasic decline probably represents release from intracellular spaces, which in this work are interpreted to be the nerve endings.

FIGURE 7

Controls: Spontaneous Efflux of ^{3}H-noradrenaline.

Upper Curve: total tritium remaining in the perfused heart.

Results shown were calculated by adding the amount of tritium found in the hearts at the end of the experiment to the amount of tritium which had been released into the perfusing fluid during each of the preceding intervals. The values are the average of 8 hearts. The corresponding data are found in Appendix II, Table 7.

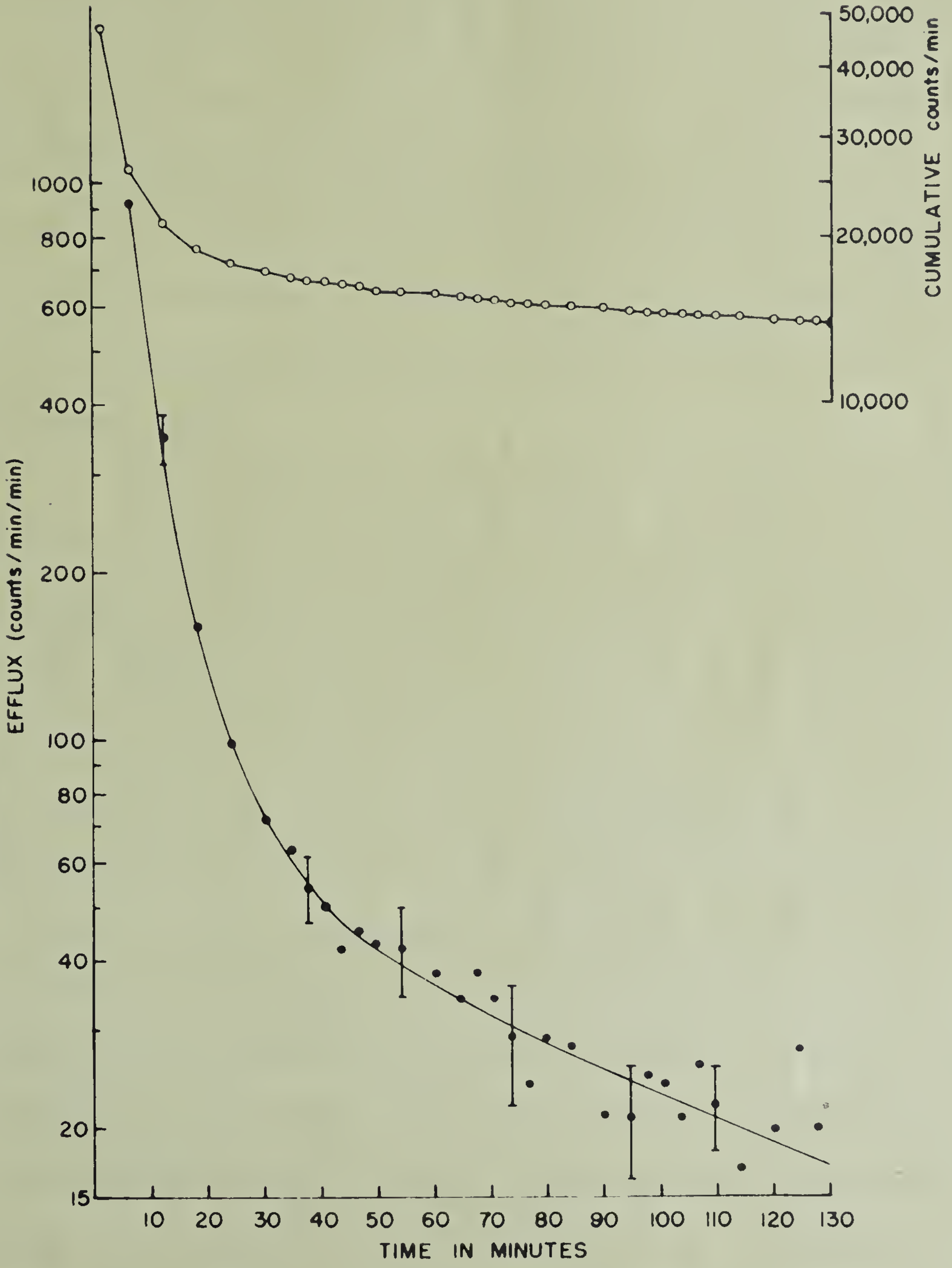
Lower Curve: rate of loss of radioactivity.

The perfusate was collected for varying intervals (3 or 6 minutes) and each point represents the mean rate of release during that collection period. The ordinate shows the mean rate of tritium release from the heart, at the midpoint of the interval, expressed as counts/min of tritium leaving the heart per minute. Each point is the average of 8 hearts. The corresponding data are found in Appendix II, Table 7.

FIGURE 7

Controls: Spontaneous Efflux of

^{3}H-Noradrenaline



(iv) Drug-Induced Release.

Since a 1 μM concentration of drug was found to give nearly a maximum increase in the rate of tritium release, all drugs were administered in this concentration. Three 1 μM doses were administered at intervals of 30 minutes and each dose period was 9 minutes. In most cases the effect of the drug had worn off after 20 minutes so that the next dose could be given. In order to estimate the change in rate of release of tritium caused by the drug, it was not satisfactory to merely take the difference in release before and at the peak of the releasing action of the drug but it was necessary to attempt to estimate the rate of spontaneous release which would have been occurring at the time of peak releases and to subtract that from the release rate at the point of maximum action.

Figure 8 shows the efflux curve of one heart from which three periods of release were induced by infusing noradrenaline for 9 minutes. To estimate the level of spontaneous release at the point of peak release, the average efflux curve plotted in Figure 7 was fitted to the initial portion of the efflux curve for this heart (i.e., fitted up to the point of drug administration). This heart contained more than the average amount of ^{3}H-noradrenaline and the efflux rate was higher than average but it was assumed that the shape of the efflux curve would be the same as the average curve in Figure 7 and that the rate of spontaneous efflux could be estimated by extending the initial curve along the rate of change curve as plotted in Figure 7. The change in rate of efflux induced by infusing noradrenaline was therefore estimated by taking the difference between the peak rate of release and the estimate of what the spontaneous rate of release would have been at the time of

the highest rate of release.

If the rate of release of ^{3}H-noradrenaline from the nerve ending is proportional to the amount present in the heart, then the rate of release at each of these periods should be a constant proportion of the amount in the heart at that time. To test this hypothesis, 4 hearts were perfused and similar estimates of the release caused by ℓ-noradrenaline were made at three infusion periods, 30 minutes apart. The data for the estimated percentage release from the 4 hearts at each of three ℓ-noradrenaline infusion periods are included in Appendix II, Table 9. It may be observed that although the average maximum release from the hearts during the three periods varied from 0.89 to 1.20% of the amount in the heart, the variation within the group of hearts during each release period was equally as large and statistical tests indicated there was no significant difference between these mean values. These experiments therefore gave 12 separate estimates, on 4 different hearts, of the releasing activity of noradrenaline which, on the average, was 1.05% of the amount present in the heart at the time of maximum release.

The method used above was applied to estimate the releasing activity of the other sympathomimetic amines and the data obtained are included in Appendix II, Table 9. With the exception of metaraminol and d-isoprenaline there was no significant difference between the mean release at each of the three different 1 μM doses. In the case of d-isoprenaline the release caused by the first and third doses were significantly different from one another although there was no significant difference between the release caused by the first and second dose and the second and third doses. The releases caused by metaraminol

were all significantly different from one another, the first release being large and equal to that caused by ℓ-noradrenaline, whereas the releases due to subsequent doses fell off sharply. It therefore appeared that metaraminol blocked its own releasing activity and should not be considered in the same class with the other drugs.

Due to the fact that some drugs are slower to come to the peak release than are others some form of standardization was sought. It was therefore decided to take the peak response occurring within the 9-minute infusion period as the point of comparison.

Table I summarizes these data and ranks the sympathomimetic amines tested in their order of potency as noradrenaline releasing agents.

FIGURE 8

Effect of ℓ-NA on the Release of Tritium
from Isolated, Perfused Rat Hearts.

Upper Curve: total tritium remaining in the perfused heart (counts/min).

Results shown were calculated by adding the amount of tritium found in the heart at the end of the experiment to the amount of tritium which had been released into the perfusing fluid during each of the preceding intervals. The values are those obtained from one heart. The corresponding data are found in Appendix II, Table 8.

Lower Curve: rate of loss of radioactivity (counts/min/min).

The dotted line shows how the control efflux curve of Figure 7 is fitted unto efflux curves of experiment in which drugs had been administered. (The curves fall together for the first 39 min.) The solid line shows the effect of infusing a 1 μM concentration of ℓ-NA at the times indicated on the abscissa. The increase in rate of release is estimated by dropping a vertical line from the peak release occurring within the 9-minute administration period, to the baseline as shown. This change in the rate of release of ^{3}H-noradrenaline is taken as a percentage of the amount in the heart at that time (obtained from the cumulative counts curve).

The values are those obtained from one heart. The corresponding data are found in Appendix II, Tables 8 and 9.

FIGURE 8

Effect of ℓ-NA on the Release of Tritium from Isolated, Perfused Rat Hearts.

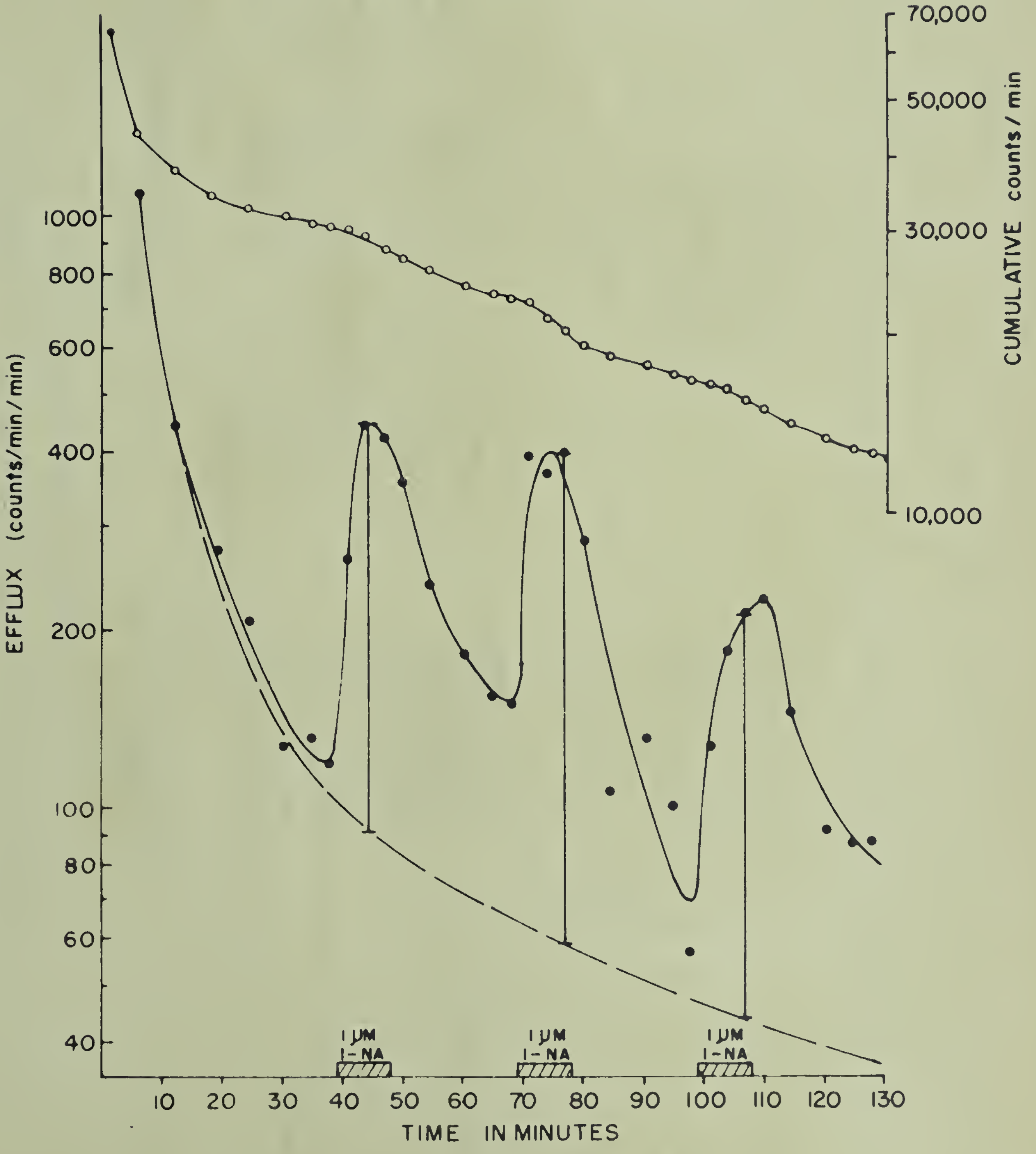


TABLE 1

Increase in the Rate of Release of Tritium Produced by Ten Sympathomimetic Amines.

	Drug	Rate (% of ^{3}HNA in Heart) ± S.E.	Significance of Difference	
1	ℓ-NA	1.05 ± 0.09	1 and 2, $0.3 < p > 0.2$;	1 and 3, $0.03 < p > 0.01$
2	Dopamine	0.89 ± 0.07	2 and 3, $0.3 < p > 0.2$;	2 and 4, $0.005 < p > 0.001$
3	p-Tyramine	0.76 ± 0.06	3 and 4, $0.3 < p > 0.2$;	3 and 5, $0.005 < p > 0.001$
4	ℓ-A	0.65 ± 0.03	4 and 5, $0.05 < p > 0.025$;	4 and 6, $p < 0.001$
5	d-NA	0.50 ± 0.04	5 and 6, $0.2 < p > 0.1$;	5 and 7, $0.1 < p > 0.05$
6	d-A	0.42 ± 0.03	6 and 7, $0.5 < p > 0.4$;	6 and 8, $0.01 < p > 0.005$
7	ℓ-INA	0.36 ± 0.07	7 and 8, $0.5 < p > 0.4$;	7 and 9, $p < 0.001$
8	Phenylethylamine	0.30 ± 0.03	8 and 9, $p < 0.001$	
9	*d-INA	-0.13 ± 0.02		

Tests	dℓ-Met	d-INA
1st	1.04 ± 0.12	-0.20 ± 0.04
2nd	0.56 ± 0.04	-0.14 ± 0.02
3rd	0.35 ± 0.07	-0.07 ± 0.02
mean	0.65 ± 0.10	-0.13 ± 0.02

* Although the 1st and 3rd doses of d-INA were significantly different, the mean of the three doses was included for comparative purposes.

The corresponding data are in Appendix II, Table 9.

(v) The Amount of ^{3}H-Noradrenaline Released.

The previous method of calculation gave only a partial picture of the drug effect, since the rate of change and the duration of action of some amines differ from others. Thus a ranking order based on the peak rate of release at an arbitrary time after starting the infusion may differ considerably from one based on the total amount of amine released during a prolonged time period.

Starting from the period before drug administration and continuing for 30 minutes, to the beginning of the next drug administration, each efflux point was calculated as a percent of the total amount of tritium remaining in the heart at that time. This was done for the three doses of each drug as well as for the controls. As the values for each separate dose period for any one drug corresponded fairly well, the average of these three dose-effect tests was taken as indicated in Appendix II, Table 10. In order to obtain a common starting point, the efflux value obtained in the period before the start of drug infusion was taken as the zero position in each case, including the controls, and this value was subtracted from each value in the series to obtain the change in the rate of release occurring in time. These values were plotted for each drug as shown in Figure 9. The areas under the curves (using the control as baseline) were determined by use of a planimeter and the values obtained were converted to figures representing the amounts of ^{3}H-noradrenaline released, which were expressed as percentages of amounts in the heart (Table II).

Values obtained are presented in Table II.

FIGURE 9

The Change in Rate of Tritium Efflux (as % of Amount in the Heart) Caused by 1 μM Doses of the Ten Sympathomimetic Amines.

The rate of tritium efflux was calculated as a percentage of the amount in the heart at the time $(\frac{\text{efflux (c/m/m)}}{\text{Cum. c/m}} \times 100)$ starting from the period before drug administration and continuing for a period of 30 minutes of which the drug was infused for the first 9 minutes. During the subsequent 21 minutes the rate of release returned to approximately the initial rate.

To show the change in the rate of efflux due to the infusion of a drug, the rate of efflux during the collection period just prior to starting the drug infusion was subtracted from each subsequent efflux rate. Each point represents the mean of three 1 μM doses on each of 4 hearts (i.e., of 12 separate tests).

The corresponding data are in Appendix II, Table 10.

FIGURE 9

The Change in Rate of Tritium Efflux (as % of Amount in the Heart) Caused by 1 μM Doses of the Ten Sympathomimetic Amines.

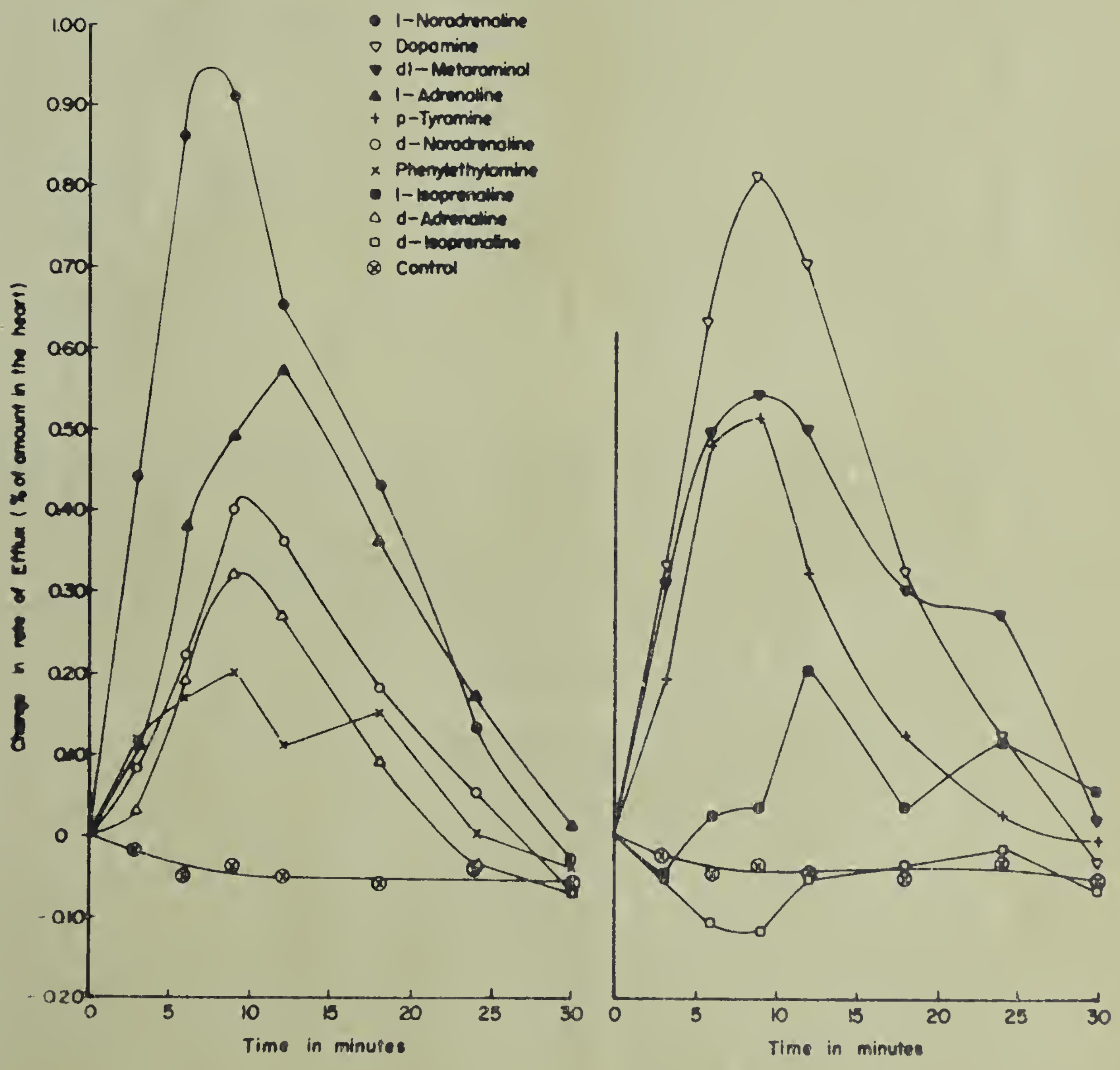


Table II

Amount of Tritium Released (Expressed as % of Amount in the Heart).

Drug	Area under Curve (sq. units) y	Amount Released $(\frac{y}{37.3} \times 30)$
ℓ-NA	17.7	14.3
Dopamine	15.6	12.6
dℓ-Met	13.8	11.2
ℓ-A	12.4	10.0
p-Tyramine	8.9	7.2
d-NA	7.5	6.1
d-A	5.4	4.4
Phenylethylamine	5.1	4.1
ℓ-INA	4.1	3.3
d-INA	- .12	-0.1

The amount of ^{3}H-noradrenaline released from the heart during a 30 minute period, expressed as a percentage of the total amount of ^{3}H-noradrenaline present in the heart, was $\frac{y}{x} \times 30$, where y = area under the curve and x = the area represented by a 1%/min release rate for 30 minutes, that is, 37.3 square units, which is 30% of the total amount in the heart.

Table III

Summary: Ranking Order of Drug Actions.

Drug	Stimulation of α-Receptors	Stimulation of Release Rate	Amount of Tritium Released	Daly (Amount of Tritium Released)	Iversen (Inhibition of ^{3}H-NA Uptake)
ℓ-A	1	4	4	2	5
ℓ-NA	2	1	1	1	3
d-A	3	6	7	5	8
dℓ-Met	4	4?	3	-	-
d-NA	4	5	6	4	7
ℓ-INA	5	7	9	-	-
Dopamine	6	2	2	7	2
p-Tyramine	7	3	5	6	4
Phenylethylamine	✓	8	8	8	6
d-INA	✓	9	10	-	-
ℓ-Met	-	-	-	3	1
dℓ-INA	-	-	-	9	9
Catecholamines with β-hydroxyl groups					
ℓ-NA	2	1	1	1	1
ℓ-A	1	2	2	2	2
d-NA	3	3	3	3	3
d-A	4	4	4	4	4
ℓ-INA	5	5	5	-	-
d-INA	-	6	6	-	-
dℓ-INA	-	-	-	5	5

✓ = no response

- = not tested

DISCUSSION

In recent years it has been suggested that sympathomimetic amines may act either by a direct effect on the receptor, or/and by an indirect action through noradrenaline release in the neighborhood of the receptor site (116, 35). It has also been recently postulated that noradrenaline released from nerve endings is rapidly removed from the vicinity of the receptors by a reuptake mechanism which transports the excess noradrenaline back across the nerve membrane for restorage (88, 99). The primary object of this project was to investigate whether the receptor pattern of the postulated noradrenaline carrier in the sympathetic nerve endings is similar to that of the α-adrenotropic receptor. In addition, we wished to determine the influence of the structural changes on the releasing activity of sympathomimetic amines. Chemorelease is probably a two phase process involving first the uptake of the releasing agent into the nerve ending, and secondly its exchange at the storage site. In this study an initial assumption was that the uptake of the sympathomimetic agent by the nerve ending was the limiting factor in release of ^{3}H-noradrenaline so that a measure of the rate of release is an indication of the structural similarity to, and affinity of the drug for, the amine carrier of the nerve ending transport system.

Within the phenylethylamine series many modifications of structure can be made without abolishing either α-receptor stimulating or noradrenaline releasing activity. For this study the drugs chosen were

mainly catecholamines, which are reported to act directly on the α-adrenotropic receptor, but in addition three drugs with more or less indirect α-receptor stimulating activity were included. In keeping with the classical studies of structure-activity relationships we wished to determine the effect of:

1. addition of hydroxyl groups to the phenylethylamine skeleton (to the benzene ring and to the β-carbon atom).
2. alterations at the amine nitrogen atom.
3. stereoisomer changes about the β-carbon atom (i.e., of the hydroxyl groups).

Traditionally, the contractile action of sympathomimetic amines in smooth muscle has been regarded as an α-receptor effect, and the relaxant action a β-receptor effect (39). It is probable, however, that both α- and β-receptors are stimulated in smooth muscle that contracts in response to sympathomimetic amines, so that response observed is the resultant of two opposing actions and the relaxant effect of the β-receptors does not become apparent until the α-receptors have been blocked. Likewise the full α-effect cannot be shown unless the subtracting β-effects are blocked. In this study, in which the interest was in the α-effect, the β-effect was blocked by propranolol, a β-blocking agent which is free from intrinsic sympathomimetic activity. Initial experiments done without the use of the β-blockade showed no difference between the response to ℓ-noradrenaline and ℓ-adrenaline but when the β-receptors were blocked the responses to ℓ-adrenaline were clearly greater than those to ℓ-noradrenaline, particularly at low dose levels. For that reason the β-blocking agent was added to the medium

during all tests of the drugs but it was found that under the conditions of the dose of the β-blocking agent used ℓ-isoprenaline gave little or no response. Therefore the doses of propranolol given with the ℓ-isoprenaline tests was increased by 100 fold, which resulted in a definite contractile response to that drug.

As the catecholamines in our drug series are direct acting agents (33), it appeared unnecessary to reserpine-treat the animals prior to use of their tissues. Our data placed p-tyramine close to the end of the series and phenylethylamine showed no action within the dose range employed. In a few experiments on tissues from reserpine treated animals, tyramine gave no response and the positions of dopamine and metaraminol in the series were not sufficiently altered to influence our conclusions, which placed the order of potency of the catecholamines used as:

ℓ-A > ℓ-NA > d-A > d-NA > ℓ-INA > Dopamine > d-INA.

Metaraminol fell in the same response range as d-A, while p-tyramine was less active than dopamine. Phenylethylamine and d-INA gave no response within the dose range tested.

Figure 2 and Table III indicate the order of potency of response of β-blocked vascular smooth muscle to this series of sympathomimetic drugs. These results are in agreement with the structure-activity reports of earlier workers in this field (29, 30, 33).

The orders of potency of the catecholamines as ^{3}H-noradrenaline releasing agents, stated either as increases in the rate of release or in the amounts released, were: ℓ-NA > Dopamine > ℓ-A > d-NA > d-A > ℓ-INA > d-INA. In stimulation of release rate p-tyramine ranked after dopamine, metaraminol was about equally effective as ℓ-A while phenylethylamine came between ℓ-INA and d-INA. As stimulators of amounts

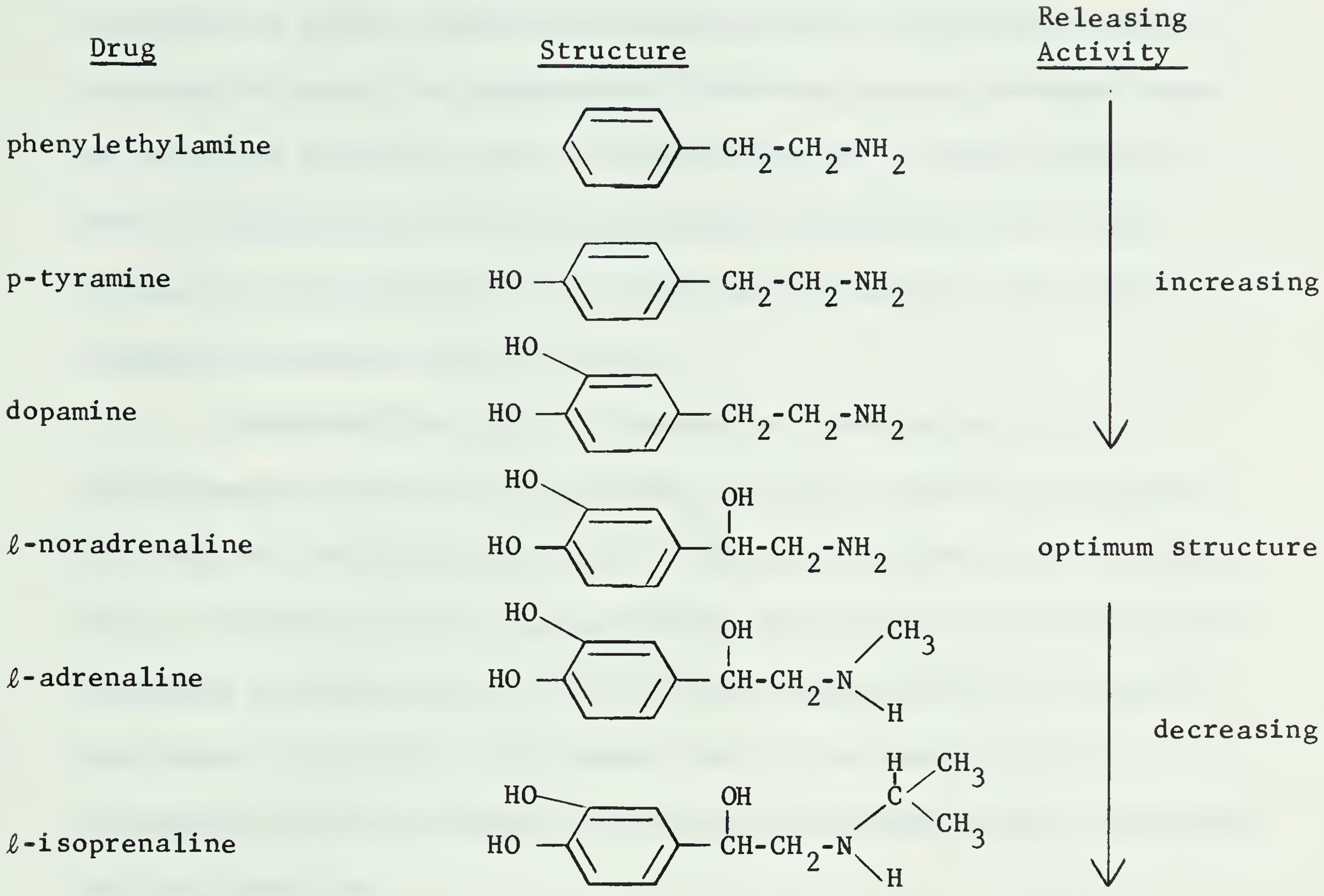
released, the order following dopamine was metaraminol, ℓ-A and then p-tyramine.

Although as indicated in Table III, a direct comparison of the ranking order of potency of these sympathomimetic agents as stimulators of α-receptors and as releasers of noradrenaline shows little correlation, there exist some noteworthy similarities in the relation of structure to these different activities. First, in both cases, a definite stereo-specificity appears in each of the isomeric pairs of catecholamines. Second, the secondary amines are less potent releasers than the primary amines and the releasing activity decreases with an increase in the size of the substituted radical. Although ℓ-A is a more potent α-receptor stimulator than ℓ-NA, further substitution of larger radicals at the amino nitrogen reduced the stimulation of the muscle. Third, by comparing phenylethylamine, p-tyramine and dopamine it may be seen that addition of a hydroxyl group to the benzene structure progressively increased the α-receptor stimulating and the releasing activity, so that the catechol structure gave optimal activity in both cases. There is also an apparent increased releasing effect of the addition of the β-hydroxyl group observed when comparing the releasing activity of dopamine and ℓ-NA. Although statistical tests indicated that these means are not significantly different, this may be due to the limitations of the measuring system and the difference in means is a true indication of difference in releasing activity. The effects of adding groups to the phenylethylamine molecule are shown in Figure 10.

Thus the three major points of structural change contribute to the increasing potency of releasing action in a manner similar to

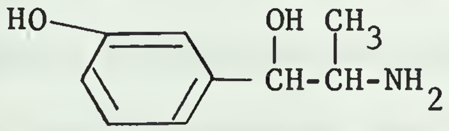
Figure 10

Influence of Structure on Releasing Activity.



If β-hydroxylation results in the formation of the dextro-isomer, the releasing activity is much reduced.

dℓ-metaraminol



The releasing activity of dℓ-metaraminol is approximately equal to that of ℓ-adrenaline. Metaraminol does not have the optimal catechol structure, but is a primary amine.

that in which they contribute to the stimulation of α-receptors. However, there are also noteworthy differences, particularly in the matter of relative potency and the degree of specificity. Changing from ℓ- to d-isomers of either adrenaline or noradrenaline alters the α-receptor stimulation potency by approximately 100-fold whereas the same change in structure results in only a 2-fold change in releasing activity. Thus the specificity of the factor controlling release from nerve endings is very much lower than those controlling the contractile response of vascular smooth muscle.

Comparing the order of potency as releasing activity of catecholamines observed in this work, with that reported by Iversen (13, 99) for inhibition of uptake of ^{3}H-noradrenaline into rat hearts, there is almost complete agreement with the exception of the reversed positions of dopamine and ℓ-NA for first ranking position. Phenylethylamine, p-tyramine and dopamine fall in the same orders of increasing potency as do the isomeric pairs of isoprenaline, adrenaline and noradrenaline.

Daly _et al._, working with mouse hearts _in vivo_ (3) also found that of the various classes of sympathomimetic amines which release ^{3}H-noradrenaline, catecholamines were the most active. Next in activity were phenolic amines followed by the nonphenolic phenylethylamines. Secondary amines were less active than primary amines and tertiary amines were inactive. In further agreement with the findings presented here, Daly _et al._ (3) also found that the ℓ-isomers of noradrenaline and adrenaline were better releasing agents than the unnatural d-isomers. In contrast to our findings these workers ranked dopamine as a less active releasing agent than ℓ-A, metaraminol and tyramine. This

discrepancy may have been due to the differences in the method of application. The open ended perfusion system used in these experiments continuously placed new drug into the system, whereas in their closed in vivo system the dopamine may have been rapidly bound, metabolized directly, and/or converted to noradrenaline and subsequently bound or metabolized, thus reducing the concentration of releasing agent presented for exchange with bound ^{3}H-noradrenaline. Isoprenaline was found to be relatively inactive as a releasing agent probably because, in contrast to noradrenaline and adrenaline for example (7), it is not exchanged and bound intracellularly in the nerves of the heart (117).

Iversen has found metaraminol to have the highest affinity for the noradrenaline uptake sites (97) whereas these experiments showed it to be a less potent releaser than ℓ-NA. However, it was found that successive doses of metaraminol showed a decreasing effect on release. Metaraminol is avidly taken up into the nerve endings (100) but may, in exchanging with the storage sites, become so firmly bound that it partially blocks the effect of a second and a third dose of metaraminol. Many sympathomimetic amines which release noradrenaline are themselves retained in storage sites as "false transmitters" (106).

A number of lipo soluble basic compounds including cocaine and desmethylimipramine block the uptake of circulating noradrenaline into nerve endings (13, 15, 16, 17). If uptake into the nerve ending must occur before the releasing agent can exchange with and cause an increase in outflow of ^{3}H-noradrenaline then these agents which block uptake should retard release.

Figure 5 shows that cocaine HCl itself has no effect on the spontaneous release rate of tritium from the isolated perfused heart but

considerably reduces the release induced by ℓ-NA. The inhibition of release by cocaine is apparently a competitive type of inhibition, for when the concentration of cocaine was increased from 10^{-6} M to 10^{-5} M only the highest dose of ℓ-NA (2 μM) showed any releasing effect. The observation that cocaine by itself had no effect upon the rate of spontaneous release is not consistent with the pump-leak theory of Brodie and Beaven (118) for the mechanism of releasing action. They propose that noradrenaline is constantly leaving the axon by passive diffusional leakage down a concentration gradient between the intra-axonal fluid and the extracellular fluid. This outflow is opposed by the noradrenaline "pump," situated in the axonal membrane, which returns the noradrenaline to the intra-axonal fluid and thus maintains a high concentration of noradrenaline in the axon. According to this hypothesis reserpine and other noradrenaline depleting drugs act by inhibiting the noradrenaline pump, and thus allowing the intracellular noradrenaline to leak out of the axons. However, if this were the case then other inhibitors of noradrenaline uptake such as cocaine and desmethylimipramine should also act as noradrenaline depleting agents.

Part of the reason no increase in rate of release is observed when the pump is blocked by cocaine could be that in the open ended perfusion system used the rate of perfusion is so high (4 ml/min) that there is normally very little opportunity for reuptake to occur. Thus the spontaneous loss of ^{3}H-noradrenaline continues at the same rate regardless of the presence of cocaine.

Desmethylimipramine blocks the uptake of noradrenaline but does not decrease the concentration of the amine in brain or in heart when administered *in vivo* (119). However, *in vitro*, high levels of

desmethylimipramine (130 μg/ml) have been found to displace some radioactivity from hearts previously perfused with labelled noradrenaline (120). In the experiments reported here, desmethylimipramine (10^{-6} M) caused a small transient increase in the efflux of tritium and when ℓ-NA was infused, as shown in Figure 6, the releasing effects of all but the highest doses of ℓ-NA (2 μM) were blocked.

Iversen (99) has reported that both cocaine and desmethylimipramine are very potent inhibitors of ^{3}H-noradrenaline uptake into isolated perfused hearts. This may be further equated with our findings that these two drugs inhibited the release of ^{3}H-noradrenaline caused by ℓ-NA. Thus it was concluded that inhibition of the uptake of the releasing agent (in this case, ℓ-NA) by the nerve ending results in inhibition of ^{3}H-noradrenaline release by the releasing agent. Hence the limiting factor in release could be the uptake of the releasing agent into the nerve ending.

The diversity of lipid-soluble amines which block the uptake of catecholamines into sympathetic nerve endings is remarkable. These include imipramine and a number of imipramine-like depressants such as ametriptyline, the phenothiazines, chlorpromazine and promazine, dibenzyline, cocaine and a number of other substances (121, 122). With this diversity, it is difficult to think that the compounds act on a specific carrier by virtue of any structural resemblance to the catecholamines.

Our data show that the limiting factor for release of ^{3}H-noradrenaline by ℓ-NA could possibly be the uptake of ℓ-NA into the nerve ending, but we cannot at present state that this may also be the case for other sympathomimetic amines. It is not known if other amines

are transported by the same carrier as that postulated for noradrenaline. Lundborg (123) has recently postulated that in isolated adrenal medullary granules, metaraminol inhibits the ATP-Mg^{++} dependent uptake of adrenaline but does not utilize this mechanism for uptake. Metaraminol is taken up by displacing the endogenous catecholamines from their storage sites. Likewise, in the nerve ending there may be more than one mechanism for uptake of amines. An interesting project to carry out in this work would be to test the releasing activity of the other amines in the presence of cocaine. This could prove especially interesting in the case of d-INA which caused an inhibition of the spontaneous release of ^{3}H-noradrenaline. It is difficult to speculate how d-INA inhibits this release. It may be altering the properties of the membrane in some way so that the ^{3}H-noradrenaline is not released.

Release of ^{3}H-noradrenaline by metaraminol is also difficult to explain in the sense that successive doses produce less and less release. Metaraminol, being resistant to monoamine oxidase, may accumulate within the nerve terminal to such an extent that it competes with the ^{3}H-noradrenaline for outward transport.

Exactly how the uptake of noradrenaline into adrenergic nerve terminals occurs remains obscure. Theories put forth (e.g., active transport, or a pump-leak system) have not as yet been proven (10, 118). Just as the mechanism of uptake remains obscure so does the mechanism of release. In order for an amine to release stored ^{3}H-noradrenaline it must first get into the nerve terminal and then exchange at the storage sites. Once the exchange has taken place, the displaced ^{3}H-noradrenaline must be transported out of the nerve terminal. Uptake into the nerve ending, exchange at the storage

site and transport out of the nerve terminal may all be mediated by some sort of carrier system. These carriers may have the same or different specificity. However, this has yet to be proven.

SUMMARY AND CONCLUSIONS

1. The contractile responses of β-receptor blocked isolated rabbit aorta tissue to increasing doses of sympathomimetic amines were plotted in the log dose-response manner and gave the typical sigmoid curves. The order of potency of these drugs studied as α-receptor stimulators was:

   ℓ-A > ℓ-NA > d-A = dℓ-Met > d-NA > ℓ-INA > Dopamine > Tyramine.

   Phenylethylamine and d-INA gave no response in the dose range tested.

2. These sympathomimetic amines were tested as releasers of ^{3}H-noradrenaline from isolated perfused rat hearts whose noradrenaline stores had been previously labelled with dℓ-^{3}H-noradrenaline. The effects of the amines on both the rate of release and the total amount of ^{3}H-noradrenaline released were studied.

   The order of potency as stimulators of an increased rate of release was:

   ℓ-NA > Dopamine > p-Tyramine > ℓ-A = dℓ-Met > d-NA > d-A >
   ℓ-INA > Phenylethylamine > d-INA.

   d-INA caused a reduction in the rate of release.

The order of potency with respect to the total amount of ^{3}H-noradrenaline released was the same as the above with the exception that following dopamine the order was:

dℓ-Met > ℓ-A > p-Tyramine > d-NA, etc.

The differences between these two orders of potency is apparently due to the fact that dℓ-Met and ℓ-A have more prolonged actions than tyramine.

3. Structure-activity relationships observed include the following:
    i. For both α-receptor stimulating and ^{3}H-noradrenaline releasing activity, an increase in the degree of phenolic hydroxylation enhanced activity. The optimal structure was the 3,4-dihydroxyphenyl group.
    ii. N-substitution decreased the ability to release. The effect was dependent on the size of the N-substituent, bulky substituents being less potent. In contrast, substitution of a methyl group slightly enhanced the activity as an α-receptor stimulator but substitution of the larger isopropyl radical decreased this activity.
    iii. β-hydroxylation to form the levo-isomer produced an increase in release, whereas β-hydroxylation to form the dextro-isomer produced a definite inhibition of release. Similarly the levo-isomers are more effective stimulators of α-receptors than are the dextro-isomers.
4. Although there were a number of similarities between the effect of structural changes on α-receptor stimulation and releasing activity, the effect of changes in the structure of the stimulating agent was very much greater on the former than on the latter. The α-receptor exhibited a very high degree of specificity whereas, in contrast, the postulated amine carrier exhibited a low degree of specificity. It was concluded that although there may be some similarity between

the receptor pattern of these two active sites, the α-receptor is much more selective and probably more complex than that of the amine carrier.

5. Cocaine and desmethylimipramine, which block the uptake of noradrenaline and other amines into the nerve endings, were found to block the release of ^{3}H-noradrenaline induced by ℓ-NA. These observations suggest that uptake of the releasing agent into the nerve ending is necessary before exchange and release can occur. Thus the affinity for the noradrenaline uptake site may be the limiting factor for release.
6. The mechanism of how release is induced by these drugs remains obscure. It may be a simple exchange process mediated by a carrier, whereby one or more molecules of drug entering the nerve ending exchanges with one or more molecules of bound noradrenaline. On the other hand the drugs may induce a receptor stimulated change in the membrane structure, analogous to that occurring at muscle membranes, which initiates a chain of events resulting in interference with the binding sites for noradrenaline and its subsequent release. In view of the low specificity and selectivity of this responding system, it appears more likely that a relatively simple exchange system is the mechanism of release.

BIBLIOGRAPHY

1. Chidsey, C. A., D. C. Harrison and E. Braunwald. Release of norepinephrine from the heart by vasoactive amines. Proc. Soc. Exp. Biol. (N.Y.), 109: 488-490, 1962.

2. Nash, C. W., E. Costa and B. B. Brodie. The actions of reserpine, guanethidine and metaraminol on cardiac catecholamine stores. Life Sci., 3: 441-449, 1964.

3. Daly, J. W., C. P. Creveling and B. Witkop. The chemorelease from mouse hearts. Structure-Activity Relationships. I. Sympathomimetic and Related Amines. J. Med. Chem., 9: 273-280, 1966.

4. Anden, N-E. On the mechanism of noradrenaline depletion by α-methylmetatyrosine and metaraminol. Acta pharmacol. et toxicol., 21: 260-271, 1964.

5. Shore, P. A. The mechanism of norepinephrine depletion by reserpine, metaraminol and related agents. The role of monoamine oxidase. Pharmacol. Rev., 18: 561-568, 1966.

6. Nash, C. W., E. Costa and B. B. Brodie. Stereospecificity in the release of ^{3}H-norepinephrine from rat hearts to D and L isomers of norepinephrine. Pharmacologist, 5: 258, 1963.

7. Axelrod, J., H. Weil-Malherbe and R. Tomchick. The physiological disposition of ^{3}H-epinephrine and its metabolite metanephrine. J. Pharmacol. Exp. Therap., 127: 251-256, 1959.

8. Dengler, H. J., H. E. Spiegel and E. O. Titus. Uptake of tritium-labelled norepinephrine in brain and other tissues of cat in vitro. Science, 133: 1072-1073, 1961.

9. Whitby, L. G., J. Axelrod and H. Weil-Malherbe. The fate of ^{3}H-norepinephrine in animals. J. Pharmacol. Exp. Therap., 132: 193-201, 1961.

10. Dengler, H. J., I. A. Michaelson, H. E. Spiegel and E. O. Titus. The uptake of labelled norepinephrine by isolated brain and other tissues of the cat. Int. J. Neuropharmacol., 1: 23-28, 1962.

11. Iversen, L. L. The uptake of noradrenaline by the isolated perfused rat heart. Brit. J. Pharmacol., 21: 523-537, 1963.

12. Lindmar, R. and E. Muscholl. Die Wirkung von Pharmaka auf die Elimination von Noradrenalin aus der Perfusionsflussigkeit und die Noradrenalinaufnahme in das isolierte Herz. Arch. exp. Path. Pharmak., 247: 469-492, 1964.

13. Iversen, L. L. Inhibition of noradrenaline uptake by drugs. J. Pharm. Pharmacol., 17: 62-64, 1965.

14. Musacchio, J. M., I. J. Kopin and V. K. Weise. Subcellular distribution of some sympathomimetic amines and their β-hydroxylated derivatives in the rat heart. J. Pharmacol. Exp. Therap., 148: 22-28, 1965.

15. Hertting, G., J. Axelrod and R. W. Patrick. Actions of cocaine and tyramine on uptake and release of ^{3}H-norepinephrine in the heart. Biochem. Pharmacol., 8: 246-248, 1961.

16. Muscholl, E. Effect of cocaine and related drugs on the uptake of noradrenaline by heart and spleen. Brit. J. Pharmacol., 16: 352-359, 1961.

17. Glowinski, J. and J. Axelrod. Inhibition of uptake of tritiated-noradrenaline in the intact rat brain by imipramine and structurally related compounds. Nature, Lond., 204: 1318-1319, 1964.

18. Matsumoto, C., E. Costa and B. B. Brodie. The interaction of tyramine and desmethylimipramine (DMI) with norepinephrine stores in rat heart. Pharmacologist, 6: 206, 1964.

19. Vulpian, M. Note sur quelques réactions propres à la substance des capsules surrénales. C. R. Acad. Sci., Paris, 43: 663-665, 1856.

20. Kohn, A. Die chromaffinen Zellen des Sympathicus. Anat. Anz., 15: 393-400, 1899.

21. Oliver, G. and E. A. Schafer. The physiological effects of extracts of the suprarenal capsules. J. Physiol. (Lond.), 18: 230-276, 1895.

22. Dakin, H. D. The synthesis of a substance allied to adrenaline. Proc. Roy. Soc., Ser. B76: 491-497, 1905.

23. Stolz, F. Synthese der wirksamen Substanz der Nebennieren. Synthetisches Suprarenin. Chemikerztg., 30: 981-982, 1906.

24. Bergström, S., U. S. von Euler and U. Hamberg. Isolation of noradrenaline from the adrenal gland. Acta Chem. Scand., 3: 305, 1949.

25. Gaddum, J. H. and F. Lembeck. The assay of substances from the adrenal medulla. Brit. J. Pharmacol., 4: 401-408, 1949.

26. Euler, U. S. von. A specific sympathomimetic ergone in adrenergic nerve fibres (sympathin) and its relations to adrenalin and noradrenalin. Acta Physiol. Scand., 12: 73-97, 1946.

27. Cannon, W. B. and A. Rosenblueth. Studies on conditions of activity in endocrine organs. XXIX. Sympathin E and Sympathin I. Amer. J. Physiol., 104: 557-574, 1933.

28. Euler, U. S. von. Identification of the sympathomimetic ergone in adrenergic nerves of cattle (Sympathin N) with laevo-noradrenaline. Acta Physiol. Scand., 16: 63-74, 1948.

29. Barger, G. and H. H. Dale. Chemical structure and sympathomimetic action of amines. J. Physiol. (Lond.), 41: 19-59, 1910.

30. Fleckenstein, A. and J. H. Burn. The effect of denervation on the action of sympathomimetic amines on the nictitating membrane. Brit. J. Pharmacol., 8: 69-78, 1953.

31. Fleckenstein, A. and H. Bass. Zum Mechanismus der Wirkungsverstärkung und Wirkungsabschwächung sympathomimetischer Amine durch Cocain und andere Pharmaka. I. Mitt.: Die Sensibilisierung der Katzen-Nickhaut für Sympathomimetika der Brenzkatechin-Reihe. Arch. exp. Path. Pharmak., 220: 143-156, 1953.

32. Fleckenstein, A. and D. Stockle. Zum Mechanismus der Wirkungsverstärkung und Wirkungsabschwächung sympathomimetischer Amine durch Cocain und andere Pharmaka. II. Mitt.: Die Hemmung der Neuro-Sympathomimetika durch Cocain. Arch. exp. Path. Pharmak., 224: 401-415, 1955.

33. Trendelenburg, U. Supersensitivity and subsensitivity to sympathomimetic amines. Pharmacol. Rev., 15: 225-276, 1963.

34. Carlsson, A., E. Rosengren, A. Bertler and J. Nilsson. Effect of reserpine on the metabolism of catecholamines. In: "Psychotropic Drugs," ed. by S. Garattini and V. Ghetti, pp. 263-272, Elsevier Publ. Co., Amsterdam, 1957.

35. Trendelenburg, U., A. Muskus, W. W. Fleming and B. Gomez Alonso de la Sierra. Modification by reserpine of the action of sympathomimetic amines in spinal cats; a classification of sympathomimetic amines. J. Pharmacol. Exp. Therap., 138: 170-180, 1962.

36. Fleming, W. W. and U. Trendelenburg. The development of supersensitivity to norepinephrine after pretreatment with reserpine. J. Pharmacol. Exp. Therap., 133: 41-51, 1961.

37. Innes, I. R. and H. W. Kosterlitz. The effects of preganglionic denervation on the responses of the nictitating membrane to sympathomimetic substances. J. Physiol. (Lond.), 124: 25-43, 1954.

38. Dale, H. H. On some physiological actions of ergot. J. Physiol. (Lond.), 34: 163-206, 1906.

39. Ahlquist, R. P. A study of adrenotropic receptors. Amer. J. Physiol., 153: 586-600, 1948.

40. Ariëns, E. J. Various types of receptors for sympathomimetic drugs. In: "Adrenergic Mechanisms," pp. 264, Ciba Found. Symp., 1960.

41. Loewi, O. Uber Humorale Ubertragbarkeit der Herznervewirkung. Pflüger's Arch. ges. Physiol., 189: 239, 1921.

42. Lissak, K. Evidence for adrenaline in adrenergic neurons. Amer. J. Physiol., 125: 765-777, 1939.

43. Goodall, McC. Studies of adrenaline and noradrenaline in mammalian heart and suprarenals. Acta Physiol. Scand., 24: suppl. 85, 1951.

44. Falck, B. Observations on the possibilities of the cellular localization of monoamines by a fluorescence method. Acta Physiol. Scand., 56: suppl. 197, 1962.

45. Blaschko, H. and A. D. Welch. Localization of adrenaline in cytoplasmic particles of the bovine adrenal medulla. Arch. exp. Path. Pharmak., 219: 17-22, 1953.

46. Euler, U. S. von and N.-Å. Hillarp. Evidence for the presence of noradrenaline in submicroscopic structures of adrenergic axons. Nature, 177: 44-45, 1956.

47. Potter, L. T. and J. Axelrod. Subcellular localization of catecholamines in tissues of the rat. J. Pharmacol. Exp. Therap., 142: 291-298, 1963.

48. Kirshner, N. Pathway of noradrenaline formation from dopa. J. Biol. Chem., 226: 821-825, 1957.

49. Potter. L. T. Storage of norepinephrine in sympathetic nerves. Pharmacol. Rev., 18: 439-451, 1966.

50. Hillarp, N.-Å. Isolation and some biochemical properties of the catecholamine granules in the cow adrenal medulla. Acta Physiol. Scand., 43: 82-96, 1958.

51. Potter, L. T. and J. Axelrod. Properties of norepinephrine storage particles of the rat heart. J. Pharmacol. Exp. Therap., 142: 299-305, 1963.

52. Weiner, N. and O. Jardetzky. A study of catecholamine nucleotide complexes by nuclear magnetic resonance spectroscopy. Arch. exp. Path. Pharmak., 248: 308-318, 1964.

53. Burack, W. R., E. Averg, P. R. Drasḱoczy and N. Weiner. The number of catecholamine storage granules in adrenal gland. Proceedings of the First International Pharmacological Meeting, 5: 85-91, 1963. Pergamon Press, N. Y.

54. Banks, P. and H. Blaschko. Chromaffin Tissue. Pharmacol. Rev., 18: 453-456, 1966.

55. Douglas, W. W. and R. P. Rubin. The mechanism of catecholamine release from the adrenal medulla and the role of calcium in stimulus-secretion coupling. J. Physiol. (Lond.), 167: 288-310, 1963.

56. Trendelenburg, U. Modification of the effect of tyramine by various agents and procedures. J. Pharmacol. Exp. Therap., 134: 8-17, 1961.

57. Crout, J. R., A. J. Muskus and U. Trendelenburg. Effect of tyramine on isolated guinea-pig atria in relation to their noradrenaline stores. Brit. J. Pharmacol., 18: 600-611, 1962.

58. Potter, L. T., J. Axelrod and I. J. Kopin. Differential binding and release of norepinephrine and tachyphylaxis. Biochem. Pharmacol., 11: 254-256, 1962.

59. Kopin, I. J., G. Hertting and E. K. Gordon. Fate of norepinephrine-^{3}H in the isolated perfused rat heart. J. Pharmacol. Exp. Therap., 138: 34-40, 1962.

60. Costa, E., R. Montanari, M. A. Beaven and B. B. Brodie. Turnover rates of norepinephrine in hearts of intact mice, rats and guinea-pigs using tritiated norepinephrine. Life Sci., 2: 232-240, 1963.

61. Chidsey, C. A. and D. C. Harrison. Studies on the distribution of exogenous norepinephrine in the sympathetic neurotransmitter stores. J. Pharmacol. Exp. Therap., 140: 217-223, 1963.

62. Potter, L. T. and J. Axelrod. Studies on the storage of norepinephrine and the effect of drugs. J. Pharmacol. Exp. Therap., 140: 199-206, 1963.

63. Axelrod, J., G. Hertting and R. W. Patrick. Inhibition of ^{3}H-norepinephrine release by monoamine oxidase inhibitors. J. Pharmacol. Exp. Therap., 134: 325-328, 1961.

64. Stjarne, L. Studies of catecholamine uptake, storage and release mechanisms. Acta Physiol. Scand., 62: suppl. 228, 1964.

65. Muscholl, E. Drugs interfering with the storage and release of adrenergic transmitters. In: G. B. Koelle, W. W. Douglas, A. Carlsson and V. Trčka (eds.), "Pharmacology of Cholinergic and Adrenergic Transmission." Oxford, 1964.

66. Campos, H. A., R. E. Stitzel and F. E. Shideman. Actions of tyramine and cocaine on catecholamine levels in subcellular fractions of isolated cat heart. J. Pharmacol. Exp. Therap., 141: 290-300, 1963.

67. Iversen, L. L. and L. G. Whitby. The subcellular distribution of catecholamines in normal and tyramine depleted mouse hearts. Biochem. Pharmacol., 12: 582-584, 1963.

68. Fischer, J. E. and S. H. Snyder. Norepinephrine in sympathetic ganglia. (In preparation--obtained through Kopin, I. J. Pharmacol. Rev., 18: 522, 1966.)

69. Euler, U. S. von and F. Lishajko. Noradrenaline release from isolated nerve granules. Acta Physiol. Scand., 51: 193-203, 1961.

70. Kopin, I. J. Biochemical aspects of release of norepinephrine and other amines from sympathetic nerve endings. Pharmacol. Rev., 18: 513-523, 1966.

71. Crout, J. R. Uptake and release of ^{3}H-norepinephrine by the guinea-pig heart in vivo. Arch. exp. Path. Pharmak., 248: 85-98, 1964.

72. Weiner, N. The Catecholamines: Biosynthesis, Storage and Release, Metabolism, and Metabolic Effects. In: "The Hormones," 4: 403-479, Acad. Press Inc., New York, 1964.

73. Kopin, I. J. and W. Bridgers. Differences in D and L norepinephrine. Life Sci., 2: 356-362, 1963.

74. Maickel, R. P., M. A. Beaven and B. B. Brodie. Implications of uptake and storage of norepinephrine by sympathetic nerve endings. Life Sci., 2: 953-958, 1963.

75. Potter, L. T., T. Cooper, V. L. Willman and E. Wolfe. Synthesis, binding, release and metabolism of norepinephrine in normal and transplanted hearts. Circulation Res., 16: 468-481, 1965.

76. Snyder, S. H., J. Glowinski and J. Axelrod. The storage of norepinephrine and some of its derivatives in brain synaptosomes. Life Sci., 4: 797, 1965.

77. Muscholl, E. Die Hemmung der Noradrenalin-Aufnahme des Herzens durch Reserpin und die Wirkung von Tyramin. Arch. exp. Path. Pharmak., 240: 234-241, 1960.

78. Strömblad, B. C. R. and M. Nickerson. Accumulation of epinephrine and norepinephrine by some rat tissues. J. Pharmacol. Exp. Therap., 134: 154-159, 1961.

79. Campos, H. A. and F. E. Shideman. Subcellular distribution of catecholamines in the dog heart. Int. J. Neuropharmacol., 1: 13-22, 1962.

80. Hertting, G., J. Axelrod and L. G. Whitby. Effect of drugs on the uptake and metabolism of ^{3}H-norepinephrine. J. Pharmacol. Exp. Therap., 151: 146-153, 1961.

81. Kopin, I. J. and E. K. Gordon. Metabolism of administered and drug released norepinephrine-7-H^3 in the rat. J. Pharmacol. Exp. Therap., 140: 207-216, 1963.

82. Siegel, J. H., J. P. Gilmore, and S. J. Sarnoff. Myocardial extraction and production of catechol amines. Circulation Res., 9: 1336-1350, 1961.

83. Lindmar, R. and E. Muscholl. Einfluss von Pharmaka auf die Elimination von Noradrenalin aus der Perfusionsflussigkeit des isolierten Herzens. Arch. exp. Path. Pharmak., 243: 347, 1962.

84. Gillespie, J. S. and S. M. Kirpekar. Removal of noradrenaline from the blood flowing through the spleen. J. Physiol. (Lond.), 169: 100P, 1963.

85. Thoenen, H., A. Huerlimann and W. Haefely. The effect of sympathetic nerve stimulation on volume, vascular resistance and norepinephrine output in the isolated perfused spleen of the cat and its modification by cocaine. J. Pharmacol. Exp. Therap., 143: 57-63, 1964.

86. Lindmar, R. and E. Muscholl. Bilanzversuche mit noradrenalin-Infusionen am perfundierten Rattenherzen. Arch. exp. Path. Pharmak., 245: 99-100, 1963.

87. Hertting, G., J. Axelrod, I. J. Kopin and L. G. Whitby. Lack of uptake of catecholamines after chronic denervation of sympathetic nerves. Nature, 189: 66, 1961.

88. Hertting, G. and J. Axelrod. Fate of tritiated noradrenaline at the sympathetic nerve-endings. Nature, 192: 172-173, 1961.

89. Levi-Montalcini, R. Growth control of nerve cells by a protein factor and its antiserum. Science, 143: 105-110, 1964.

90. Sjöquist, F., E. Titus, I. A. Michaelson, P. Taylor and K. C. Richardson. Uptake and metabolism of d,ℓ-norepinephrine-7-H^3 in tissues of immunosympathectomized mice and rats. Life Sci., 4: 1125-1133, 1965.

91. Wolfe, D. E., L. T. Potter, K. C. Richardson and J. Axelrod. Localizing tritiated norepinephrine in sympathetic axons by electron microscopic autoradiography. Science, 138: 440-442, 1962.

92. Marks, B. H., T. Samorajski and E. J. Webster. Radioautographic localization of norepinephrine-H^3 in the tissues of mice. J. Pharmacol. Exp. Therap., 138: 376-381, 1962.

93. Hamberger, B., T. Malmfors, K.-A. Norberg and Ch. Sacks. Uptake and accumulation of catecholamines in peripheral adrenergic neurons of reserpinized animals, studied with a histochemical method. Biochem. Pharmacol., 13: 841-844, 1964.

94. Fischer, J. E. and I. J. Kopin. Evidence for intra- and extraneuronal reserpine resistant norepinephrine pools. Fed. Proc., 23: 349, 1964.

95. Beaven, M. and R. P. Maickel. Stereoselectivity of norepinephrine storage sites in heart. Biochem. Biophys. Res. Comm., 14: 509-513, 1964.

96. Iversen, L. L. The uptake of catecholamines at high perfusion concentrations in the rat isolated heart: A novel catechol amine uptake process. Brit. J. Pharmacol., 25: 18-33, 1965.

97. Iversen, L. L. Inhibition of noradrenaline uptake by sympathomimetic amines. J. Pharm. Pharmacol., 16: 435-436, 1964.

98. Burgen, A. S. V. and L. L. Iversen. The inhibition of noradrenaline uptake by sympathomimetic amines in the rat isolated heart. Brit. J. Pharmacol., 25: 34-49, 1965.

99. Iversen, L. L. Inhibition of noradrenaline uptake by drugs. In: "Advances in Drug Research," 2: 1-46, 1965.

100. Shore, P. A., D. Busfield and H. S. Alpers. Binding and release of metaraminol: Mechanism of norepinephrine depletion by α-methyl-m-tyrosine and related agents. J. Pharmacol. Exp. Therap., 146: 194-199, 1964.

101. Euler, U. S. von and F. Lishajko. Release of noradrenaline from adrenergic transmitter granules by tyramine. Experientia, 16: 376, 1960.

102. Schümann, H. J. and A. Philippu. Untersuchungen zum Mechanismus der Freisetzung von Brenzcatechinaminen durch Tyramin. Arch. exp. Path. Pharmak., 241: 273-280, 1961.

103. Euler, U. S. von and F. Lishajko. Effect of reserpine on the release of catecholamines from isolated nerve and chromaffin cell granules. Acta Physiol. Scand., 52: 137-145, 1961.

104. Stjärne, L. Storage particles in noradrenergic tissues. Pharmacol. Rev., 18: 425-432, 1966.

105. Crout, J. R. and P. A. Shore. Release of metaraminol (aramine) from the heart by sympathetic nerve stimulation. Clin. Res., 12: 180, 1964.

106. Fischer, J. E., W. D. Horst and I. J. Kopin. β-hydroxylated sympathomimetic amines as false neurotransmitters. Brit. J. Pharmacol., 24: 477-484, 1965.

107. Brodie, B. B., C. C. Chang and E. Costa. On the mechanism of action of guanethidine and bretylium. Brit. J. Pharmacol., 25: 171-178, 1965.

108. Costa, E. Interactions of drugs with adrenergic neurons. Pharmacol. Rev., 18: 577-597, 1966.

109. Udenfriend, S. and P. Zaltzman-Nirenberg. On the mechanism of norepinephrine depletion by aramine. Life Sci., 3: 695-702, 1964.

110. Lineweaver, H. and D. Burk. The determination of enzyme dissociation constants. J. Am. Chem. Soc., 56: 658-666, 1934.

111. Eadie, G. S. On the evaluation of the constants V_m and K_m in enzyme reactions. Science, 116: 688, 1952.

112. Dowd, J. E. and D. S. Riggs. A comparison of estimates of Michaelis-Menten Kinetic Constants from various linear transformations. J. Biol. Chem., 240: 863-869, 1965.

113. Mounter, L. A. and M. E. Turner. The evaluation of Michaelis Constants and maximal velocity kinetic studies of enzymic reactions. Enzymologia, 25: 225-230, 1962.

114. Callingham, B. A. and R. Cass. The determination of catecholamines in biological materials. In: The Clinical Chemistry of Monoamines, ed. by H. Varley and A. H. Gowenlock, pp. 19-30, Elsevier Publ. Co., Amsterdam, 1963.

115. Callingham, B. A. and R. Cass. A modification of the butanol extraction method for fluorimetric assay of catecholamines in biological materials. J. Pharm. Pharmacol., 15: 699-700, 1963.

116. Burn, J. H. and M. J. Rand. The action of sympathomimetic amines in animals treated with reserpine. J. Physiol. (Lond.), 144: 314, 1958.

117. Hertting, G. The fate of ^{3}H-isoproterenol in the rat. Biochem. Pharmacol., 13: 1119-1128, 1964.

118. Brodie, B. B. and M. A. Beaven. Neurochemical transducer systems. Med. Exp., 8: 320-351, 1963.

119. Susler, F., J. Watts and B. B. Brodie. On the mechanism of depressant action of imipramine-like drugs. Ann. N. Y. Acad. Sci., 96: 279-286, 1962.

120. Titus, E. O., N. Matussek, H. E. Spiegel and B. B. Brodie. The effects of desmethylimipramine on uptake of dℓ-norepinephrine-7-H^3 in the heart. J. Pharmacol. Exp. Therap., 152: 469-477, 1966.

121. Fawaz, G. and J. Simaan. Cardiac noradrenaline stores. Brit. J. Pharmacol., 20: 569-578, 1963.

122. Shore, P. A. Release of serotonin and catecholamines by drugs. Pharmacol. Rev., 14: 531-550, 1963.

123. Lundborg, P. Uptake of Metaraminol by the Adrenal Medullary Granules. Acta Physiol. Scand., 67: 423-429, 1966.

APPENDIX I

Preparation of Solutions

All solutions used were prepared in distilled water which had been passed through a deionizer.

Krebs-Bicarbonate Solution:

Concentrated salt solution -

Sodium Chloride (NaCl)	82.60 grams
Potassium Chloride (KCl)	4.22 "
Calcium Chloride ($CaCl_2$)	3.36 "
Potassium Phosphate (KH_2PO_4)	1.94 "
Magnesium Sulfate ($MgSO_4 \cdot 7H_2O$)	3.50 "

This mixture of salts was dissolved in water to produce a total volume of one litre.

Normal Krebs-bicarbonate solution was prepared by mixing 100 mℓ of the concentrated solution, 900 mℓ of distilled water, 2 grams of glucose and 5 grams of sodium bicarbonate contained in 192 mℓ of distilled water. The pH of this solution is 7.6. When aerated with 5% CO_2 and 95% O_2 mixture, the pH is 7.35 to 7.40. This solution contains the following millimolar concentrations:

NaCl 118 mM, KCl 4.7 mM, $CaCl_2$ 2.5 mM,
KH_2PO_4 1.2 mM, $MgSO_4 \cdot 7H_2O$ 1.2 mM,
$NaHCO_3$ 25 mM, glucose 10 mM.

Acid Krebs solution was prepared by mixing 100 mℓ of the concentrated salt solution with 900 mℓ of distilled water. (pH = 5.5)

Preparation of dℓ-^{3}H-noradrenaline for Infusion:

The dℓ-^{3}H-noradrenaline used in these experiments contained 43 μg noradrenaline per mℓ (S.A. = 4.27 c/mM).

The solution was first diluted 1:100 in 0.01N HCl (= 0.43 μg/mℓ).

7.5 mℓ of this solution was diluted to 40 mℓ with acid Krebs (Krebs containing no glucose or bicarbonate). The concentration of this solution was 80 ng noradrenaline/mℓ.

This radioactive solution was infused into the apparatus at a rate of 0.22 mℓ/min where it was further diluted with Krebs solution which was perfusing the hearts at 4 mℓ/min. The final concentration of noradrenaline reaching the heart was 4.2 ng/mℓ/min.

Stock Solutions of Drugs:

All the sympathomimetic amines were made up in 0.01N HCl at a 2.0 mM concentration.

For experiments the stock solution was diluted 1:100 in acid Krebs (strength = 0.02 mM).

When infused at a rate of 0.22 mℓ/min and the solution further diluted with Krebs solution being perfused at 4 mℓ/min the final concentration of the solution reaching the heart was 1 μM.

Acidified Salt-Saturated n-Butanol:

20 grams of NaCl and 1 mℓ of concentrated HCl was added to 500 mℓ n-butanol and shaken for 1 hour.

APPENDIX II

Abbreviations:

NA = Noradrenaline

A = Adrenaline

Met = Metaraminol

INA = Isoprenaline

DMI = Desmethylimipramine

TABLE 1

Responses of Vascular Smooth Muscle to ℓ-Noradrenaline in the Presence of Propranolol (10 ng/ml of bath volume).

Dose	3×10^{-4} mM		6×10^{-4} mM		3×10^{-3} mM		6×10^{-3} mM		Estimated Maximum	
	Resp *	% Max **	Resp *	% Max **	Resp *	% Max **	Resp *	% Max **	Lineweaver-Burke Method	Eades Method
	12	33	14	38	24	67	33	92	36	36
	4	10	5	12	23	58	25	62	40	36
	9	24	11	30	19	51	31	84	37	39
	11	29	15	40	29	76	33	87	38	37
	17	32	25	47	43	81	45	85	53	51
	4	10	9	22	27	68	27	68	40	43
	4	10	9	22	20	50	30	75	40	40
	11	31	18	50	31	86	33	92	36	37
	18	46	26	67	36	92	37	95	39	40
	5	24	9	43	17	81	20	95	21	22
	9	38	14	58	22	92	22	92	24	25
	15	40	24	63	35	92	37	97	38	41
	19	41	31	67	43	94	44	96	46	48
$\overline{x}$		28		43		76		86	37.5	38

* Responses are in mm deflection and each value represents the mean of 4 tissues. (10 mm = 1 gm tension)

** Values were calculated as a % of the maximum response as obtained by the Lineweaver-Burke method.

TABLE 2

Responses of Vascular Smooth Muscle to Symapthomimetic Amines in the Presence of Propranolol (10 ng/ml of bath volume).

Dose:	$3x10^{-4}$mM		$6x10^{-4}$mM		$3x10^{-3}$mM		$6x10^{-3}$mM		$3x10^{-2}$mM		$6x10^{-2}$mM		$1.2x10^{-1}$mM		$3x10^{-1}$mM		$6x10^{-1}$mM		Estimated Maximum Response to ℓ-NA
DRUG	Resp	% Max	Resp	% Max	Resp	% Max	Resp	% Max	Resp	% Max	Resp	% Max	Resp	% Max	Resp	% Max	Resp	% Max	
ℓ-NA*		28		43		76		86											
ℓ-A	15	42	18	50	28	78	33	92											36
	14	37	16	42	30	79	33	87											38
		39		46		78		89											
d-A					-	0	9	25	26	72	22	61							36
					3	8	13	35	27	71	22	58							38
						4		30		72		59							
dℓ-Met					4	10	9	22	30	75	33	82							40
					2	6	10	28	27	75	29	81							36
					2	10	5	24	14	67	17	81							21
						9		25		72		81							
d-NA					-	-	4	11	20	56	20	56							36
					-	-	5	13	21	55	19	50							38
								12		56		53							
ℓ-INA									19	41	33	72	41	89					46
Dopamine							3	8	21	54	28	72	34	87					39
							2	4	20	44	30	65	38	83					46
								6		49		68		85					
Tyramine											6	11			15	28	21	40	53
											4	10			13	33	18	46	39
												11				31		43	

Note: The responses to ℓ-isoprenaline were in the presence of 1 μg/mℓ of propranolol.

*Values for ℓ-NA were obtained from Table 1.

TABLE 3

The Effect of Successively Increasing Doses of ℓ-NA, ℓ-A and dℓ-Met on the Release of Tritium from Isolated, Perfused Rat Hearts.
(Each value represents the mean of 4 hearts.)

Dose		CONTROL	ℓ-NA		ℓ-A		dℓ-Met	
of Drug	Time (min)	CPM x AF /Min	CPM x AF /Min	Cum CPM	CPM x AF /Min	Cum CPM	CPM x AF /Min	Cum CPM
	0	-		159,192		128,245		104,142
	3	9107	8920	132,432	9179	100,709	8374	79,018
	6	1954	2332	125,436	2927	94,927	1875	73,392
	9	1197	1197	121,845	1124	88,181	1086	70,133
	12	835	896	119,158	705	86,066	696	68,045
	15	612	740	116,937	568	84,362	545	66,409
	18	472	630	115,048	501	82,858	408	65,185
	21	464	545	113,412	406	81,639	315	64,241
	24	386	434	112,109	321	80,675	315	63,297
	27	328	412	110,872	307	79,755	257	62,526
	30	308	346	109,835	297	78,864	217	61,874
	33	289	328	108,851	243	78,134	199	61,278
	36	246	333	107,853	204	77,521	177	60,746
	39	293	337	106,842	234	76,820	204	60,134
	42	246	301	105,938	224	76,148	195	59,549
	45	265	279	105,100	307	75,228	151	59,097
0.5 μM	48	195	421	103,836	262	74,381	293	58,219
	51	191	913	101,096	404	71,169	479	56,783
	54	148	1157	97,625	526	71,592	510	55,253
1.0 μM	57	176	1414	93,382	739	69,373	621	53,391
	60	203	1503	88,730	613	66,935	589	51,622
	63	203	1428	84,590	812	64,447	554	49,959
2.0 μM	66	183	1476	80,161	881	61,855	559	48,283
	69	183	1361	76,078	779	59,519	528	46,700
	72	160	1326	72,101	774	57,198	483	45,250
	75	156	846	69,561	613	55,358	426	43,973
	78	156	674	67,539	521	53,796	390	42,803
	81	195	585	65,783	457	52,424	341	41,779

(continued next page)

TABLE 3 (continued)

Dose		CONTROL	ℓ-NA		ℓ-A		dℓ-Met	
of Drug	Time (min)	CPM x AF /Min	CPM x AF /Min	Cum CPM	CPM x AF /Min	Cum CPM	CPM x AF /Min	Cum CPM
	84	187	483	64,333	433	51,125	270	40,968
	87	179	434	63,030	360	50,045	266	40,120
	90	179	368	61,926	302	49,140	222	39,505
	93	176	293	61,048	287	48,279	208	38,880
	96	129	288	60,183	219	47,622	195	38,295
	99	129	284	59,332	224	46,950	182	37,750

TABLE 4

Dose-Response Curves of ℓ-NA, dℓ-Met and ℓ-A on Release of Tritium from Rat Hearts.

(Each value represents the mean of 4 hearts and basic data were obtained from Table 3.)

	ℓ-NA			dℓ-Met			ℓ-A		
Dose of Drug	Rate of Efflux of ^{3}HNA	Change in Rate of Efflux	Mean Response	Rate of Efflux of ^{3}HNA	Change in Rate of Efflux	Mean Response	Rate of Efflux of ^{3}HNA	Change in Rate of Efflux	Mean Response
	0.26	0.00		0.25	0.00		0.30	0.00	
0.5 µM	0.40	0.14		0.49	0.24		0.35	0.05	
	0.88	0.62	0.75	0.82	0.57	0.60	0.54	0.24	0.33
	1.14	0.88		0.89	0.64		0.72	0.42	
1.0 µM	1.45	1.19	1.35	1.12	0.87	0.84	1.03	0.73	0.74
	1.61	1.35		1.10	0.85		0.88	0.58	
	1.60	1.34		1.07	0.82		1.21	0.91	
2.0 µM	1.74	1.48	1.45	1.12	0.87	0.81	1.37	1.07	0.98
	1.69	1.43		1.09	0.84		1.26	0.96	
	1.74	1.48		1.03	0.78		1.30	1.00	
	1.17	0.91		0.94	0.69		1.07	0.77	
	0.97	0.71		0.89	0.64		0.94	0.64	

The rate was calculated as a percentage of the amount in the heart at the time:

$$\left(\frac{\text{Efflux (c/m/m)}}{\text{c/m in heart at that time}} \times 100\right)$$

TABLE 5

The Effect of Cocaine HCl on the Release of Tritium Caused by ℓ-NA.

(Each value represents the mean of 4 hearts.)

Dose of ℓ-NA	Time (in min)	ℓ-NA (Control) Efflux (c/m/m)	ℓ-NA + Cocaine HCl 10^{-6}M Efflux (c/m/m)	ℓ-NA + Cocaine HCl 10^{-5}M Efflux (c/m/m)
	3	10,863	7,290	11,379
	6	2,488	1,887	3,163
	9	1,554	1,131	1,755
	12	1,008	825	1,101
	15	767	707	940
	18	646	693	802
	21	538	484	481
	24	442	401	485
	27	400	319	529
	30	425	*278	*407
	33	379	331	273
	36	354	280	308
	39	329	262	329
	42	308	303	290
	45	271	280	256
0.5 μM	48	513	217	286
	51	876	406	221
	54	1,004	415	238
1.0 μM	57	1,238	602	247
	60	1,192	698	217
	63	1,142	760	251
2.0 μM	66	1,175	906	295
	69	1,238	873	342
	72	1,200	747	347
	75	821	556	325
	78	671	398	295
	81	546	325	303
	84	517	304	290
	87	450	356	299
	90	363	226	256
	93	363	271	256
	96	363	253	225
	99	350	196	208

*The cocaine HCl was added to the perfusion fluid at this time and its administration was continued until end of experiment.

TABLE 6

The Effect of Desmethylimipramine HCl on the Release of Tritium Caused by ℓ-NA.
(Each value represents the mean of 4 hearts.)

Dose of ℓ-NA	Time (min)	ℓ-NA (control) Efflux (c/m/m)	ℓ-NA + DMI 10^{-6}M Efflux (c/m/m)
	3	9138	8204
	6	1788	1958
	9	1067	1094
	12	713	779
	15	469	518
	18	410	453
	21	320	387
	24	286	360
	27	286	306
	30	239	* 270
	33	256	225
	36	230	343
	39	265	306
	42	222	333
	45	213	378
0.5 μM	48	183	357
	51	196	176
	54	253	162
1.0 μM	57	418	180
	60	563	167
	63	704	180
2.0 μM	66	772	212
	69	828	281
	72	806	253
	75	542	248
	78	435	243
	81	418	230
	84	308	203
	87	257	153
	90	243	162
	93	183	162
	96	175	162
	99	183	135

*DMI 10^{-6} M added to the perfusion fluid at this time and its administration was continued until end of experiment.

TABLE 7

Controls: Spontaneous Efflux of ^{3}H-noradrenaline

(Each value represents the mean of 8 hearts.)

Time (in min)	c/m in Heart	c/m in Heart at Midpoint of period (a)	Efflux c/m/m (b)	% $\frac{b}{a}$ x 100
0	47,768	35,660	7035	19.72
3	26,662	23,720	923	3.89
9	21,125	20,270	353	1.74
15	19,005	18,520	160	0.86
21	18,047	17,740	99	0.56
27	17,452	17,236	72	0.42
33	17,019	16,924	64	0.38
36	16,828	16,747	54	0.32
39	16,665	16,591	50	0.30
42	16,516	16,454	42	0.26
45	16,392	16,324	45	0.28
48	16,256	16,191	43	0.27
51	16,126	15,999	42	0.26
57	15,872	15,759	38	0.24
63	15,645	15,594	34	0.22
66	15,543	15,486	38	0.25
69	15,429	15,379	34	0.22
72	15,329	15,263	29	0.19
75	15,196	15,183	24	0.16
78	15,169	15,103	29	0.19
81	15,036	14,972	28	0.19
87	14,908	14,842	21	0.14
93	14,775	14,744	21	0.14
96	14,713	14,676	25	0.17
99	14,639	14,603	24	0.16
102	14,567	14,536	21	0.14
105	14,504	14,466	26	0.18
108	14,427	14,394	22	0.15
111	14,360	14,309	17	0.12
117	14,257	14,196	20	0.14
123	14,138	14,096	28	0.20
126	14,053	14,024	20	0.14
129	13,994			

TABLE 8

Effect of ℓ-NA on the Release of Tritium from Isolated, Perfused Rat Hearts.

(Each value represents 1 heart.)

Dose of ℓ-NA	No. of Min	Sample No.	c/m in 1 ml Effluent	x	Aliquot Factor	=	c/m x Aliquot Factor	Cum. c/m	$\frac{\text{c/m x Aliquot Factor}}{\text{No. of Min}}$
	-	-	-		-		12,410*	12,410	-
	3	32	20		13.2		264	12,674	88
	3	31	20		13.2		264	12,938	88
	6	30	21		26.4		554	13,492	92
	6	29	33		26.4		871	14,363	145
	3	28	52		13.2		686	15,049	229
1 μM	3	27	48		13.2		634	15,683	211
	3	26	42		13.2		554	16,237	185
	3	25	29		13.2		383	16,620	128
	3	24	13		13.2		172	16,792	57
	3	23	23		13.2		304	17,096	101
	6	22	30		26.4		792	17,888	132
	6	21	24		26.4		634	18,522	106
	3	20	64		13.2		845	19,367	282
1 μM	3	19	91		13.2		1,201	20,568	400
	3	18	83		13.2		1,096	21,664	365
	3	17	90		13.2		1,188	22,852	396
	3	16	34		13.2		449	23,301	150
	3	15	35		13.2		462	23,763	154
	6	14	45		26.4		840	24,603	180
	6	13	54		26.4		1,426	26,029	238
	3	12	81		13.2		1,069	27,098	356
1 μM	3	11	97		13.2		1,280	28,378	427
	3	10	102		13.2		1,346	29,724	449
	3	9	61		13.2		805	30,529	268
	3	8	27		13.2		356	30,885	119
	3	7	30		13.2		396	31,281	132

*This value is c/m left in the heart at the end of the experiment.

(Continued on next page)

TABLE 8 (continued)

Dose of ℓ-NA	No. of Min	Sample No.	c/m in 1 ml Effluent	x Aliquot Factor	= c/m x Aliquot Factor	Cum. c/m	$\frac{\text{c/m x Aliquot Factor}}{\text{No. of Min}}$
	6	6	29	26.4	766	32,047	128
	6	5	47	26.4	1,241	33,288	207
	6	4	62	26.4	1,637	34,925	273
	6	3	99	26.4	2,614	37,539	436
	6	2	248	26.4	6,547	44,086	1,091
	3	1	1,695	13.2	22,374	66,460	7,458

TABLE 9

The Increase in the Rate of Release of Tritium from Isolated Perfused Rat Hearts by Ten Sympathomimetic Amines. (Each value represents one heart.)

Drug	Dose	Heart No.	Weight of Heart (gm)	c/m in Heart at End of Expt.	Release Rate $c/m^2/gm$			c/m/gm in Heart at Peak	Release as % of Amt. in Heart	Significance of Difference
					Peak	Base	Change			
ℓ-NA	1 µM 1st dose	1	0.887	12,410	506	103	403	33,511	1.20	1st and 2nd dose $p > 0.5$
		2	0.806	17,060	596	161	435	44,000	0.99	
		3	0.893	13,490	504	134	370	31,196	1.19	
		4	0.932	13,990	370	112	258	30,216	0.85	
									$\bar{x}$ = 1.06 ± 0.09	
	1 µM 2nd dose	1			451	61	384	23,188	1.66	2nd and 3rd dose $p > 0.2$
		2			387	112	275	31,775	0.87	
		3			454	92	362	24,195	1.50	
		4			237	74	163	21,082	0.77	
									$\bar{x}$ = 1.20 ± 0.22	
	1 µM 3rd dose	1			238	50	188	16,966	1.11	1st and 3rd dose $p > 0.2$
		2			239	83	156	24,938	0.63	
		3			232	66	173	17,450	0.99	
		4			197	56	141	16,907	0.83	
									$\bar{x}$ = 0.89 ± 0.10	
									$\bar{x}$ = 1.05 ± 0.09	

Continued on next page

TABLE 9 (continued)

Drug	Dose	Heart No.	Weight of Heart (gm)	c/m in Heart at End of Expt.	Release Rate $c/m^2/gm$			c/m/gm in Heart at Peak	Release as % of Amt. in Heart	Significance of Difference
					Peak	Base	Change			
d-NA	1 μM 1st dose	1	0.895	22,000	322	112	210	36,198	0.58	1st and 2nd dose $p > 0.7$
		2	0.922	26,900	295	103	192	40,681	0.47	
		3	0.965	18,840	253	109	144	31,523	0.46	
		4	0.796	12,780	211	89	122	23,995	0.51	
									$\bar{x} = 0.51 \pm 0.03$	
	1 μM 2nd dose	1			221	69	152	30,764	0.49	2nd and 3rd dose $p > 0.8$
		2			187	56	131	35,358	0.37	
		3			273	64	209	26,817	0.78	
		4			113	54	59	20,415	0.29	
									$\bar{x} = 0.48 \pm 0.11$	
	1 μM 3rd dose	1			182	48	134	26,583	0.50	1st and 3rd dose $p > 0.9$
		2			191	38	153	31,154	0.49	
		3			200	46	154	22,183	0.69	
		4			97	39	58	17,481	0.33	
									$\bar{x} = 0.50 \pm 0.08$	
									$\bar{x} = 0.50 \pm 0.04$	

Continued on next page

TABLE 9 (continued)

Drug	Dose	Heart No.	Weight of Heart (gm)	c/m in Heart at End of Expt.	Release Rate c/m^2/gm			c/m/gm in Heart at Peak	Release as % of Amt. in Heart	Significance of Difference
					Peak	Base	Change			
d-A	1 μM 1st dose	1	0.967	33,930	307	140	167	48,202	0.35	1st and 2nd dose $p > 0.05$
		2	0.896	28,900	320	128	192	45,417	0.42	
		3	1.064	27,580	150	64	86	32,541	0.26	
		4	0.977	25,650	243	85	158	38,994	0.41	
									$\overline{x} = 0.36 \pm 0.04$	
	1 μM 2nd dose	1			263	71	192	42,252	0.45	2nd and 3rd dose $p > 0.9$
		2			270	70	200	39,762	0.50	
		3			165	39	126	30,083	0.42	
		4			207	54	153	34,387	0.44	
									$\overline{x} = 0.45 \pm 0.02$	
	1 μM 3rd dose	1			209	64	145	37,703	0.38	1st and 3rd dose $p > 0.4$
		2			233	65	168	35,438	0.47	
		3			105	35	70	27,613	0.25	
		4			243	48	195	29,357	0.66	
									$\overline{x} = 0.44 \pm 0.09$	
									$\overline{x} = 0.42 \pm 0.03$	

Continued on next page

TABLE 9 (continued)

Drug	Dose	Heart No.	Weight of Heart (gm)	c/m in Heart at End of Expt.	Release Rate $c/m^2/gm$			c/m/gm in Heart at Peak	Release as % of Amt. in Heart	Significance of Difference
					Peak	Base	Change			
ℓ-A	1 μM 1st dose	1	0.909	20,180	488	165	323	44,881	0.72	1st and 2nd doses $p > 0.4$
		2	0.740	16,590	419	156	263	42,683	0.62	
		3	0.980	14,360	246	76	170	25,894	0.66	
		4	0.712	15,980	446	125	341	38,707	0.88	
									$\bar{x}$ = 0.72 ± 0.06	
	1 μM 2nd dose	1			333	112	221	35,116	0.63	2nd and 3rd dose $p > 0.1$
		2			282	109	173	33,610	0.51	
		3			204	57	147	20,547	0.72	
		4			320	92	228	31,376	0.73	
									$\bar{x}$ = 0.65 ± 0.05	
	1 μM 3rd dose	1			293	98	195	27,830	0.70	1st and 3rd dose $p > 0.1$
		2			234	99	135	26,477	0.51	
		3			133	49	84	16,911	0.50	
		4			230	81	149	25,932	0.57	
									$\bar{x}$ = 0.57 ± 0.05	
									$\bar{x}$ = 0.65 ± 0.03	

Continued on next page

TABLE 9 (continued)

Drug	Dose	Heart No.	Weight of Heart (gm)	c/m in Heart at End of Expt.	Release Rate $c/m^2/gm$			c/m/gm in Heart at Peak	Release as % of Amt. in Heart	Significance of Difference
					Peak	Base	Change			
d-INA	1 μM 1st dose	1	0.996	25,340	40	76	- 36	28,839	-0.12	1st and 2nd doses $p > 0.2$
		2	0.784	27,670	70	179	-109	35,020	-0.31	
		3	0.828	33,150	104	163	- 59	41,284	-0.14	
		4	0.777	26,850	99	169	- 70	33,821	-0.21	
									$\overline{x}$ = -0.20 ± 0.04	
	1 μM 2nd dose	1			20	53	- 33	27,279	-0.12	2nd and 3rd doses $p > 0.02$
		2			65	121	- 56	32,515	-0.17	
		3			80	106	- 26	37,469	-0.07	
		4			55	112	- 57	30,167	-0.19	
									$\overline{x}$ = -0.14 ± 0.02	
	1 μM 3rd dose	1			24	39	- 15	26,220	-0.06	1st and 3rd doses $p > 0.02$
		2			84	92	- 8	30,654	-0.03	
		3			66	79	- 13	35,186	-0.04	
		4			45	82	- 37	28,125	-0.13	
									$\overline{x}$ = -0.07 ± 0.02	
									$\overline{x}$ = -0.13 ± 0.02	

Continued on next page

TABLE 9 (continued)

Drug	Dose	Heart No.	Weight of Heart (gm)	c/m in Heart at End of Expt.	Release Rate $c/m^2/gm$			c/m/gm in Heart at Peak	Release as % of Amt. in Heart	Significance of Difference
					Peak	Base	Change			
ℓ-INA	1 μM 1st dose	1	1.034	23,690	226	145	81	42,067	0.19	1st and 2nd doses $p > 0.2$
		2	0.975	18,960	198	133	65	39,364	0.17	
		3	0.981	20,660	246	123	123	35,140	0.35	
		4	0.909	18,600	128	110	18	30,232	0.06	
									$\overline{x} = 0.19 \pm 0.06$	
	1 μM 2nd dose	1			412	102	310	35,259	0.88	2nd and 3rd doses $p > 0.1$
		2			359	91	268	31,577	0.85	
		3			178	77	101	29,435	0.34	
		4			142	80	62	26,405	0.23	
									$\overline{x} = 0.58 \pm 0.17$	
	1 μM 3rd dose	1			212	87	125	27,705	0.42	1st and 3rd doses $p > 0.5$
		2			173	78	95	23,185	0.41	
		3			135	67	68	24,397	0.28	
		4			89	66	23	22,498	0.10	
									$\overline{x} = 0.30 \pm 0.08$	
									$\overline{x} = 0.36 \pm 0.07$	

Continued on next page

TABLE 9 (continued)

Drug	Dose	Heart No.	Weight of Heart (gm)	c/m in Heart at End of Expt.	Release Rate $c/m^2/gm$			c/m/gm in Heart at Peak	Release as % of Amt. in Heart	Significance of Difference
					Peak	Base	Change			
Dopamine	1 μM 1st dose	1	0.929	39,280	600	75	525	61,059	0.86	1st and 2nd doses $p > 0.5$
		2	1.115	17,860	298	53	245	26,345	0.93	
		3	0.763	22,800	523	118	405	44,480	0.91	
		4	0.900	22,900	662	85	577	43,284	1.33	
									$\bar{x} = 1.01 \pm 0.11$	
	1 μM 2nd dose	1			411	50	361	53,490	0.67	2nd and 3rd doses $p > 0.4$
		2			228	30	198	22,336	0.89	
		3			418	79	339	37,786	0.90	
		4			471	45	426	35,058	1.22	
									$\bar{x} = 0.92 \pm 0.11$	
	1 μM 3rd dose	1			361	45	316	46,462	0.68	1st and 3rd doses $p > 0.1$
		2			133	26	107	19,135	0.56	
		3			279	76	203	33,453	0.61	
		4			378	40	338	29,217	1.16	
									$\bar{x} = 0.75 \pm 0.14$	
									$\bar{x} = 0.89 \pm 0.07$	

Continued on next page

TABLE 9 (continued)

Drug	Dose	Heart No.	Weight of Heart (gm)	c/m in Heart at End of Expt.	Release Rate $c/m^2/gm$			c/m/gm in Heart at Peak	Release as % of Amt. in Heart	Significance of Difference
					Peak	Base	Change			
Tyramine	1 μM 1st dose	1	0.885	28,070	438	113	325	47,974	0.68	1st and 2nd doses $p > 0.5$
		2	0.906	22,430	489	121	368	53,537	0.69	
		3	0.910	21,910	319	88	231	37,603	0.61	
		4	1.042	21,600	322	92	230	30,360	0.76	
									$\bar{x}$ = 0.69 ± 0.10	
	1 μM 2nd dose	1			334	61	273	41,636	0.66	2nd and 3rd doses $p > 0.1$
		2			394	59	335	47,389	0.71	
		3			250	44	206	31,355	0.66	
		4			220	41	179	25,510	0.70	
									$\bar{x}$ = 0.68 ± 0.01	
	1 μM 3rd dose	1			270	51	219	35,967	0.61	1st and 3rd doses $p > 0.2$
		2			322	50	272	34,361	0.79	
		3			382	37	345	27,019	1.28	
		4			157	36	121	11,802	1.02	
									$\bar{x}$ = 0.93 ± 0.15	
									$\bar{x}$ = 0.76 ± 0.06	

Continued on next page

TABLE 9 (continued)

Drug	Dose	Heart No.	Weight of Heart (gm)	c/m in Heart at End of Expt.	Release Rate $c/m^2/gm$			c/m/gm in Heart at Peak	Release as % of Amt. in Heart	Significance of Difference
					Peak	Base	Change			
dℓ-Met	1 μM 1st dose	1	0.836	8,630	192	53	139	20,053	0.69	1st and 2nd doses $p > 0.001$
		2	0.902	10,440	426	122	304	24,160	1.26	
		3	0.907	16,370	473	101	372	34,623	1.07	
		4	0.986	17,140	501	127	374	32,838	1.13	
									$\overline{x} = 1.04 \pm 0.12$	
	1 μM 2nd dose	1			110	32	78	16,247	0.48	2nd and 3rd doses $p > 0.02$
		2			173	81	92	17,424	0.53	
		3			243	75	168	26,116	0.64	
		4			212	74	138	23,434	0.59	
									$\overline{x} = 0.56 \pm 0.04$	
	1 μM 3rd dose	1			72	30	42	14,265	0.29	1st and 3rd doses $p > 0.001$
		2			98	54	44	13,830	0.32	
		3			168	50	118	20,637	0.57	
		4			139	50	89	19,404	0.46	
									$\overline{x} = 0.41 \pm 0.07$	
									$\overline{x} = 0.65 \pm 0.10$	

Continued on next page

TABLE 9 (continued)

Drug	Dose	Heart No.	Weight of Heart (gm)	c/m in Heart at End of Expt.	Release Rate $c/m^2/gm$			c/m/gm in Heart at Peak	Release as % of Amt. in Heart	Significance of Difference
					Peak	Base	Change			
Phenyl-ethyl-amine	1 μM 1st dose	1	1.247	26,350	131	68	63	26,601	0.24	1st and 2nd doses $p > 0.5$
		2	0.935	15,260	119	59	60	20,884	0.29	
		3	0.988	16,090	107	28	79	23,862	0.33	
		4	1.075	27,530	207	67	140	32,814	0.43	
									$\bar{x} = 0.32 \pm 0.04$	
	1 μM 2nd dose	1			107	33	74	23,909	0.31	2nd and 3rd doses $p > 0.5$
		2			84	28	56	18,267	0.31	
		3			66	18	48	18,622	0.26	
		4			116	33	83	29,396	0.28	
									$\bar{x} = 0.29 \pm 0.01$	
	1 μM 3rd dose	1			138	27	111	22,088	0.50	1st and 3rd doses $p > 0.4$
		2			52	24	28	17,072	0.16	
		3			42	11	31	17,201	0.18	
		4			105	33	72	27,274	0.26	
									$\bar{x} = 0.28 \pm 0.08$	
									$\bar{x} = 0.30 \pm 0.03$	

Continued on next page

TABLE 10

The Increase in Release Rate of Tritium (% of the Amount in the Heart) Caused by Ten Sympathomimetic Amines. (Each value represents the mean of 4 hearts.)

Drug	Dose	Time (min)	0	3	6	9	12	18	24	30
ℓ-NA	1st		0.48	0.82	1.24	1.39	1.12	0.97	0.72	0.55
	2nd		0.55	1.21	1.39	1.34	1.36	1.16	0.56	0.37
	3rd		0.37	0.70	1.05	1.12	0.88	0.62	0.52	0.41
		$\bar{x}$	0.47	0.91	1.23	1.28	1.12	0.90	0.60	0.44
	change*		0.00	0.44	0.86	0.91	0.65	0.43	0.13	-0.03
Dop-amine	1st		0.23	0.25	0.73	1.08	1.20	0.78	0.41	0.15
	2nd		0.15	0.62	0.97	1.03	0.77	0.42	0.24	0.19
	3rd		0.19	0.67	0.83	0.85	0.67	0.30	0.22	0.16
		$\bar{x}$	0.19	0.51	0.81	0.99	0.88	0.50	0.29	0.17
	change		0.00	0.32	0.62	0.80	0.69	0.31	0.10	-0.02
p-Tyr-amine	1st		0.39	0.75	1.00	0.97	0.76	0.72	0.48	0.46
	2nd		0.46	0.64	0.87	0.81	0.69	-.46	0.37	0.42
	3rd		0.42	0.45	0.84	1.01	0.76	0.44	0.34	0.34
		$\bar{x}$	0.42	0.61	0.90	0.93	0.74	0.54	0.40	0.41
	change		0.00	0.19	0.48	0.51	0.32	0.12	-0.02	-0.01
dℓ-Met	1st		0.47	1.02	1.36	1.42	1.42	1.23	0.90	0.56
	2nd		0.56	0.74	0.87	0.81	0.83	0.57	0.72	0.39
	3rd		0.39	0.58	0.55	0.72	0.57	0.52	0.50	0.41
		$\bar{x}$	0.44	0.75	0.93	0.98	0.94	0.74	0.71	0.45
	change		0.00	0.31	0.49	0.54	0.50	0.30	0.27	0.01
ℓ-A	1st		0.43	0.58	0.90	0.98	1.05	0.83	0.69	0.47
	2nd		0.47	0.62	0.84	0.95	0.96	0.78	0.66	0.47
	3rd		0.47	0.56	0.78	0.92	1.08	0.86	0.53	0.46
		$\bar{x}$	0.46	0.57	0.84	0.95	1.03	0.82	0.63	0.47
	change		0.00	0.11	0.38	0.49	0.57	0.36	0.17	0.01

---

*Change due to drug is equal to total release less release at zero time.

TABLE 10 (continued)

Drug	Dose	Time (min)	0	3	6	9	12	18	24	30
d-NA	1st		0.32	0.51	0.57	0.77	0.63	0.41	0.35	0.30
	2nd		0.30	0.34	0.48	0.64	0.68	0.50	0.38	0.27
	3rd		0.27	0.30	0.51	0.69	0.66	0.36	0.31	0.27
		$\bar{x}$	0.30	0.38	0.52	0.70	0.66	0.42	0.35	0.28
	change		0.00	0.08	0.22	0.40	0.36	0.12	0.05	-0.02
d-A	1st		0.43	0.33	0.41	0.61	0.67	0.41	0.26	0.23
	2nd		0.23	0.27	0.54	0.62	0.52	0.38	0.22	0.22
	3rd		0.22	0.37	0.50	0.60	0.50	0.36	0.26	0.22
		$\bar{x}$	0.29	0.32	0.48	0.61	0.56	0.38	0.25	0.22
	change		0.00	0.03	0.19	0.32	0.27	0.09	-0.04	-0.07
ℓ-INA	1st		0.55	0.52	0.46	0.52	0.61	0.61	0.71	0.56
	2nd		0.56	0.56	0.80	0.72	0.97	0.53	0.85	0.66
	3rd		0.66	0.53	0.56	0.63	0.78	0.71	0.54	0.70
		$\bar{x}$	0.59	0.54	0.61	0.62	0.79	0.62	0.70	0.64
	change		0.00	-0.05	0.02	0.03	0.20	0.03	0.11	0.05
Phenyl-ethyl-amine	1st		0.25	0.40	0.51	0.51	0.42	0.37	0.26	0.20
	2nd		0.20	0.34	0.35	0.34	0.30	0.21	0.22	0.18
	3rd		0.18	0.21	0.28	0.38	0.23	0.19	0.14	0.14
		$\bar{x}$	0.21	0.32	0.38	0.41	0.32	0.36	0.21	0.17
	change		0.00	0.11	0.17	0.20	0.11	0.15	0.00	-0.04
d-INA	1st		0.40	0.35	0.24	0.19	0.32	0.36	0.37	0.27
	2nd		0.27	0.21	0.16	0.17	0.22	0.19	0.23	0.20
	3rd		0.20	0.15	0.13	0.16	0.19	0.20	0.21	0.18
		$\bar{x}$	0.29	0.24	0.18	0.17	0.24	0.25	0.27	0.22
	change		0.00	-0.05	-0.11	-0.12	-0.05	-0.04	-0.02	-0.07
CONTROL	1st		0.32	0.30	0.26	0.28	0.27	0.26	0.34	0.25
	2nd		0.25	0.22	0.19	0.16	0.19	0.19	0.14	0.17
	3rd		0.17	0.16	0.14	0.18	0.15	0.12	0.14	0.14
		$\bar{x}$	0.25	0.23	0.20	0.21	0.20	0.19	0.21	0.19
	change		0.00	-0.02	-0.05	-0.04	-0.05	-0.06	-0.04	-0.06

B29866